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Endothelin-1 Gene Polymorphism with Interleukin-1β Expression in Infertile Iraqi Women under In Vitro Fertilization Program

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Abstract

Endometrial receptivity is the limiting phase in the success of IVF treatment, Invasion of human trophoblast cells to the endometrial is regulated by many factors such as EDN1 and IL-1β. This study aims to verify the relationship between polymorphism and gene expression of EDN1 and IL-1β genes with embryo implantation in infertile women under the IVF program. The peripheral blood samples were collected on the day of embryo transfer an hour after embryo transfer, after approval from 60 women, and divided according to the outcome of embryo implantation outcomes, the failure implantation group included 35 women, and the success implantation group included 25 women. The results of sequencing revealed two genetic variants in the EDN1 gene, SNP rs2070699 showed no association with the embryo implantation outcome in infertility women according to Fisher probability. SNP rs2070699 shows a higher frequency of genotypes GG and TT than the GT genotype in the study groups. Variant No.7196T>G on exon 2 shows that the TT genotype had the highest frequency in the success group, while the TG genotype recorded the highest frequency in the failure group. The results of EDN1 and IL-1β gene expression fold change showed non-significant differences between groups. The correlation between EDN1, and IL-1β gene expression showed a significant positive relationship. In conclusion, Genetic variants of the SNPs rs2070699 and No.7196 in EDN1 do not affect the gene expression level in infertility women under the IVF program. EDN1 and IL-1β gene expression have a significant positive relationship in the luteal phase and both are upregulated in women with successful implantation.

Keywords: EDN1 gene, EDN1 gene expression, IL-1β gene, Polymorphism, Infertile Iraqi Women, IVF program.

Introduction

Since medication rarely succeeds in helping infertile women conceive, in vitro fertilization (IVF) is the preferred method of conception in infertile women[1]. In Vitro Fertilization (IVF) is a therapeutic procedure that has revolutionized infertility treatment. In this process, the egg is fertilized in the laboratory, and the resulting embryo is replaced in the uterus [2]. The implantation window is a crucial point for the embryo implant during reproductive cycle [3]. Failure in embryo implantation greatly limits the success of IVF treatments, despite the development of IVF technology the failure rate is still high[4,5]. Various factors such as growth factors and steroid hormones play critical roles in embryo implantation and affect this process [6]. Moreover, many Iraqi studies about IVF improvement

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emphasize that many genetic factors affect IVF outcomes, and recommend studying genetic variations and expression of many genes that directly affect endometrium and implantation success [2,7-10]. In other words, IVF became within the last decades a preferred solution to delay having a child in the Iraq community.

Endothelin (EDN), a 21-amino acid family of genes, has been implicated in both nonvascular and vascular smooth muscle contraction, as well as the uterus smooth muscles. It is known that EDNs are critical contributions to the event of implantation[11]. EDN1 also known as ET-1 is one of the potent vasoconstrictors secreted from endothelial cells, and it has two receptors EDNRA and EDNRB, on the external surface of the vascular smooth muscle cells of blood vessels, vessels of placental stem villi and villus trophoblast, EDN1 is involved in the specific trophoblast functions and regulation of the fetoplacental circulation[12-14]. The EDN1 was mapped to 6p24.1, it contains 5 exons, coding for a 2026-nucleotide mRNA[15,16]. Women with certain single nucleotide polymorphisms of gene *EDN1* have an increased risk of spontaneous abortion in the first trimester[17]. In the reproductive cycle, EDN1 has an important role[18], Human luteal cells are expressed on EDN, which inhibits basal and human chorionic gonadotropin (hCG)-induced progesterone production through the EDNRA[19]. The gene expression of EDN1 increasing in the ewe uterine lumen at the blastocyst expansion period before and at the implantation time[20]. Analysis of mRNA life span by using actinomycin D demonstrates that pre-pro-endothelin-1 mRNA (ppEDN1mRNA) has a short intracellular half-life of about 15 min[21]. In the endometrium, cytokine production and action play necessary roles during the complex process of embryo implantation and development [22]. The *IL-1β* gene, 7029 bp long, is located on chromosome 2q14.1 and contains seven exons and six introns encoded IL-1β protein with 269 amino acids[23,24]. IL-1β exerts a variety of biological effects and is one of the cytokines that is involved in embryo-maternal communication; its regulation of leptin secretion, in turn, activates matrix metalloproteinases which results in an increased cytotrophoblast invasion[25,26]. At the time of embryo implantation, the endometrium undergoes decidualization, that important for a successful pregnancy, decidual secretions contain high levels of pro-invasive factors such as IL-1β[27]. The current study aims to improve the outcome of the IVF program by studying the relationship among the IVF program within embryo transfer day, EDN1 genetic variation, and expression of EDN1 and IL-1β.

Materials and Methods

This comparative study was designed for infertility Iraqi women under the IVF program. Blood samples were collected after approval from (60) women in age between (20-45 years) at Al Nada Medical Center, and Rooh Alhayat Center for the Treatment of Infertility, during the period from February 2022 to August 2022. The peripheral blood samples were collected within an hour after embryo transfer. The blood sample was divided into 2 ml in an EDTA tube, and 250µl was added to 750µl GENEzol reagent, then stored at -20ºC. The samples were divided according to the outcome of the embryo implantation into two groups; the failure implantation group included 35 women, and the success implantation group included 25 women. The approval number of the ethics committee was 9557 in 2022 from the Baghdad Health Department.

DNA extraction and PCR primer

DNA extraction kits (Geneaid, Taiwan) were used to extract total genomic DNA from the whole blood, and the DNA was stored at -20˚C for further use. The polymerase chain reaction was accomplished in a reaction mixture 25µl, DNA template 5µl, Forward and Revers primers mix 2µl, free nuclease distal water 13µl, 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 position 7092 to 7740 bp represented as exon2 and intron2 used NCBI/ Primer designing tool F: 5`-GAAACCCACTCCCAGTCCAC -3`, R: 5`-AGCAAAGGAAATCCGGGCTC-3` with product size 649bp. The PCR reaction program is shown in Table 1. The PCR products and ladder markers (100 bp) were measured by electrophoresis. The bands were pictured on the UV train illuminator. The PCR product Foreword and reverse were sent for standard sequencing using ABI3730XL, an automated DNA sequence, by Macrogen Corporation (Korea). and analyzed by using Blast in NCBI and BioEdit sequence alignment editor computer program used for sequence analysis.

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Table 1. PCR amplification program for the gene at the region represented as exon2 and intron2.

Steps	Temperature (C)	Time	No. of cycles
Initial denaturation	95	5 min	1
Denaturation	94	30 sec	
Annealing	61	30 sec	35
Extension	72	40 sec	
Final extension	72	5 min	1

RNA extraction and cDNA synthesis

The extraction of RNA was done by using the GENzolTM TriRNA Pure Kit (Geneaid, Taiwan). Complementary DNA (cDNA) was synthesized using the AccuPower® RocketScriptTM RT PreMix kit and Oligo dT as a primer from Bioneer Company, Korea. The procedure was performed in reaction (20µl) and then inserted into the thermal cycler under the reaction condition in Table 2. Quantitative Real-Time PCR (qRT–PCR) was performed using AccuPower® GreenStarTM qPCR PreMix and a specific primer Designed by the second author for EDN-1 gene was F:5`-CATTTGGGTCAACACTCCCG-3`, R:5`-AGTGGAGCCAGCGCTAATGA-3`, product size 75bp and IL-1β gene was F:5`-CCTTGCTGTAGTGGTGGTCG-3`, R:5`-TGATGTCAAAGCATGGTTCCTG-3`, product size 144bp, also used housekeeping gene junctional cadherin complex regulator (JHY) primer F:5`-GTCCAGGGGTATTACAGGCAA-3`, R:5`-TCAGGAATCAGCCCAAGACG-3` product size (118bp). The PCR reaction was carried out with a total volume of 25 µl and completely mixed by exispin (5 cycles, each cycle 10 sec)), then samples were s placed in an Exicycler TM 96 device according to the program in Table 3. The levels of gene expression were quantified by measuring the threshold cycle (Ct). The fold change of target gene expression was calculated by 2^the -ΔΔCT equation[28].

Table 2. Program of cDNA synthesis.

Step	Temperature	Time
Primer annealing (oligo dT)	37ºC	10min
cDNA synthesis	42ºC	60 min
Heat inactivation	95ºC	5 min

Table 3. qRT–PCR program.

Step	Condition	Cycle
Pre-Denaturation	95°C, 3 min	1
Denaturation	95°C, 30 sec	
Annealing extension	55°C, 20 sec	40
Detection (Scan)		
Melting	-	1

Statistical Analysis

Hardy-Weinberg equilibrium (H.W.E) was tested by using the Gene-Calc- bioinformatic tool[29]. Computer program WINPEPI version 11.65 was used to analyze the statistical significance of the P-value, which was calculated with the Fisher exact test and Odd Ratio. Statistical analysis of data was performed using SPSS version 23, to detect the result of different issues on the parameters of the study. Used a t-test to significantly compare between means.

Results and Discussion

Genotyping results

The region exon2 and intron2 (from position 7092 to 7740) of the EDN1 gene were amplified under optimum conditions by using a specific primer, then amplified segment with product size 649bp measured by electrophoresis as shown in Fig. 1. The alignment of the query (sequence results) and sbjct (gene sequence on NCBI) (Table 4) show registered single nucleotide polymorphism (SNP) in two positions; SNP rs2070699 G>T, and a new position in nucleotide No.7196 T>G.

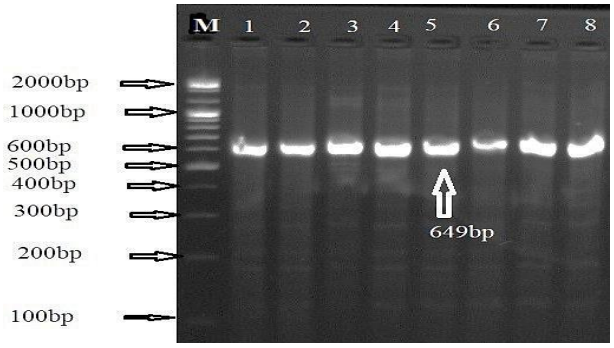

Figure 1. Gel electrophoresis for PCR product of EDN1 gene (649bp) lane 1-8 with the DNA ladder Marker (100bp) on agarose gel concentration (2%) in (70 volt/cm^2, 1 hour). M: Ladder Marker.

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Table 4. The sequencing ID, Identities, and Score of variants for exon2 and intron2 of EDN1 gene.

Accession ID:	Identities	Gaps	Score	Expected	Range
NG_016196.1	600 /605 (99%)	3/605 (0%)	1086 bits (588)	0.0	7136 to 7740

SNP rs2070699 G>T

Genetic polymorphism of the SNP rs2070699 G>T at a locus (7244) intron 2 variant of the EDN1 gene as shown in the chromatogram Fig. 2 and the blast Fig. 3 on chr6:12294025, was observed in three genotypes GG, GT, TT in the study groups. The results as seen in Table 5 indicate that the homozygous genotype GG showed a high frequency of 43% in the failure implantation group compared to the success implantation group of 32%. The heterozygous genotype GT exhibited lower frequency in the two study groups indeed 20% and 17% in the success and failure groups, respectively. While the other homozygous genotype TT showed a higher frequency 48% in the success group than in the failure group 40%. The G allele frequency in the failure and success groups was 0.51 and 0.42, respectively. While the T allele frequency was higher in the success group 0.58 than in the failure group 0.49. The results showed significant differences between observed and expected frequency in the two study groups, and the observed deviated from H.W.E. This result can lead to a conclusion that this departure in H.W.E. may be an indicator that this locus is undergoing evolutionary selection in the study sample from the Iraqi population.

Table 5. Expected genotypes and alleles frequencies of SNP rs2070699 using HWE.

Groups / SNP rs2070699 G>T Genotypes		GG	GT	TT	G	T	P-value
Failure Implantation Group	Observed no (%).	15 (43%)	6 (17%)	14 (40%)	0.51	0.49	0.00053 **
	Expected no (%).	9.26 (26%)	17.49 (50%)	8.26 (24 %)			
Success Implantation Group	Observed no (%).	8 (32%)	5 (20%)	12 (48%)	0.42	0.58	0.0129*
	Expected no (%).	4.41 (17.64%)	12.18 (48.72%)	8.41 (33.64%)			
P-value	--	0.144 NS	0.763 NS	0.694 NS		--	

** (P≤0.01), * (P≤0.05), NS: Non-Significant.

From the statistical analysis which appears in Table 6, the odd ratio (OR) of the GG genotype was 1.59 with a value of a CI 95% (confidence interval) between 0.55-4.58 and shows an etiological fraction of 0.16. While the OR of the heterozygous genotype GT was 0.83 with a CI value between 0.23-3.02 and shows a preventive fraction of 0.034. The other homozygous genotype TT OR was 0.72 with a CI between 0.26-2.00 and shows a Preventive fraction of 0.133. The G allele was recorded as an etiological fraction of 0.163 with an OR was 1.46 and a CI value between 0.71 - 3.02. While the T allele was recorded as a preventive fraction of 0.183 with an OR was 0.68 and a CI value between 0.33 - 1.41. These results showed no significant differences between the two groups of the study under the fishers' exact probability.

Table 6. The statistical comparison between genotypes in groups studied of SNP rs2070699.

SNP rs2070699 G>T Genotyps	OR	Preventive Fraction / or Etiological Fraction	Fisher's Probability	CI 95%
GG	1.59	0.16	0.432 NS	0.55 to 4.58
GT	0.83	0.034	1.000 NS	0.23 to 3.02
TT	0.72	0.133	0.603 NS	0.26 to 2.00
Allele distribution				
G	1.46	0.163	0.356 NS	0.71 to 3.02
T	0.68	0.183	0.356 NS	0.33 to 1.41

NS: Non-significant. OR: Odd Ratio. CI: confidence interval

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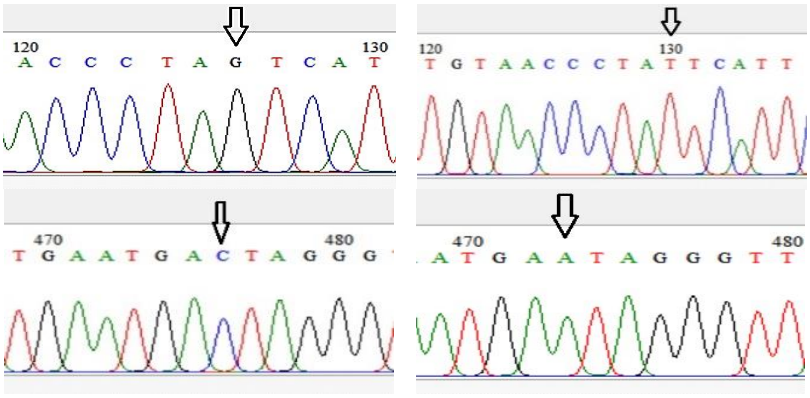

A **B**

Figure 2. DNA sequencing chromatogram which shows the SNP rs2070699 G>T. A: The arrow points to the common genotype GG/ B: the arrow points to the substitution nucleotide G/T in the forward and C/A in the reverse.

```
Query: H220906-005_E19_A27_AF.ab1 Query ID: lcl|Query_10053 Length: 1051

>Homo sapiens endothelin 1 (EDN1), RefSeqGene on chromosome 6
Sequence ID: NG_016196.1 Length: 13899
Range 1: 7136 to 7740

Score:1055 bits(571), Expect:0.0,
Identities:595/606(98%),  Gaps:3/606(0%), Strand: Plus/Plus

Query  84    AGTTGGGTCAACACTCCCGAGTAAGTCTCTAGAGGGCATTGTAACCCTAGTCATTCATTA  143
             | ||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  7195  ATTTGGGTCAACACTCCCGAGTAAGTCTCTAGAGGGCATTGTAACCCTAGTCATTCATTA  7254
```

A: Forward

```
Query: H220711-001_J06_A24_AR.ab1 Query ID: lcl|Query_4859 Length: 646
>Homo sapiens endothelin 1 (EDN1), RefSeqGene on chromosome 6
Sequence ID: NG_016196.1 Length: 13899
Range 1: 7090 to 7711
Score:1085 bits(587), Expect:0.0,
Identities:613/625(98%),  Gaps:3/625(0%), Strand: Plus/Minus

Query 427   AAAAGAAACTCTAAAACTGGGCTCCAGTGGAGCCAGCGCTAATGAATGACTAGGGTTACA 486
            ||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
Sbjct 7293  AAAAGAAACTCTAAAACTGGGCTCCAGTGGAGCCAGCGCTAATGAATGACTAGGGTTACA 7234
```

A:Reverse

```
Query: H220628-005_E17_A4_AF.ab1 Query ID: lcl|Query_3831 Length: 988

>Homo sapiens endothelin 1 (EDN1), RefSeqGene on chromosome 6
Sequence ID: NG_016196.1 Length: 13899
Range 1: 7147 to 7545

Score:608 bits(329), Expect:4e-172,
Identities:376/399(94%),  Gaps:1/399(0%), Strand: Plus/Plus

Query  92    ACTCCCGAGTAAGTCTCTAGAGGGCATTGTAACCCTATTCATTCATTAGCGCTGGCTCCA  151
             |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||
Sbjct  7207  ACTCCCGAGTAAGTCTCTAGAGGGCATTGTAACCCTAGTCATTCATTAGCGCTGGCTCCA  7266
```

B:Forward

```
Query: H220711-001_P06_A27_AR.ab1 Query ID: lcl|Query_2587 Length: 630

>Homo sapiens endothelin 1 (EDN1), RefSeqGene on chromosome 6
Sequence ID: NG_016196.1 Length: 13899
Range 1: 7093 to 7701

Score:1081 bits(585), Expect:0.0,
Identities:602/610(99%),  Gaps:1/610(0%), Strand: Plus/Minus

Query  434   AAAACTGGGCTCCAGTGGAGCCAGCGCTAATGAATGAATAGGGTTACAATGCCCTCTAGA  493
             |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||
Sbjct  7281  AAAACTGGGCTCCAGTGGAGCCAGCGCTAATGAATGACTAGGGTTACAATGCCCTCTAGA  7222
```

B: Reverse

Figure 3. DNA sequence alignment chromatogram on NCBI blast which shows the SNP rs2070699 G>T located at genomic location 7244 on the intron 2 of the EDN1 gene chr6:12294025. A: points to the common of nucleotide G in the forward and C in the reverse/ B: points to the substitution nucleotide G/T in the forward and C/A in the reverse.

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Polymorphism No.7196 T>G

Genetic polymorphism of the variant No.7196 T>G, exon 2 variant of the EDN1 gene as shown in the chromatogram Fig. 4 and the blast Fig. 5 chr6:12293977, was observed as two genotypes (TT and GT) while the other homozygotes genotype was absent in the two study groups. The results in Table 7 show the TT genotype frequency is higher at 72% in the success group than the failure group at 57%. While the heterozygous genotype TG showed the highest frequency 43% in the failure group compared to the success group 28%. The allele T had a higher frequency of 0.79, 0.86 than the G allele 0.21, 0.14 in both the failure and success study groups, respectively. This distribution is consistent with H.W.E. at P≤0.01.

Table 7. Expected genotypes and alleles frequencies N0.7196 using HWE.

Groups/ No. 7196 T>G Genotype		TT	TG	GG	T	G	P-value
Failure Implantation Group	Observed no (%)	20 (57%)	15 (43%)	0	0.79	0.21	0.272 NS
	Expected no (%)	21.61 (62%)	11.79 (33%)	1.61 (5%)			
Success Implantation Group	Observed no (%)	18 (72%)	7 (28%)	0	0.86	0.14	0.718 NS
	Expected no (%)	18.49 (74%)	6.02 (24%)	0.49 (2%)			
P-value	--	0.745 NS	0.0881 NS	1.00 NS	--		

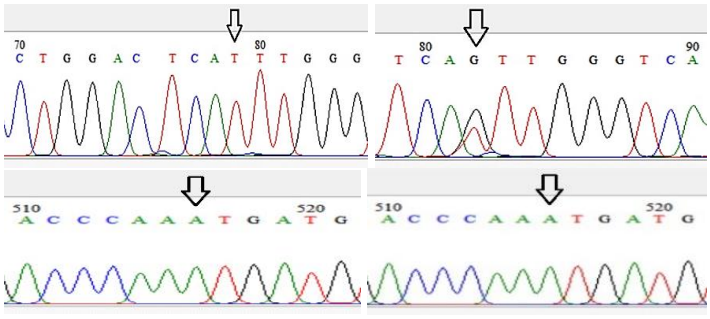
NS: Non-significant.

The statistical analysis as indicated in Table 8 shows OR of the homozygous genotype TT was 0.52 and recorded as a preventive fraction of 0.347. While the OR of the heterozygous genotype TG was 1.93 and recorded as an etiological fraction of 0.206, the T allele was recorded as a preventive fraction of 0.347 and the OR was 0.60, while the G allele was recorded as an etiological fraction of 0.128 and OR was 2.05. These results showed no significant differences between the two groups of the study under the fishers' exact probability.

Table 8. The statistical comparison between genotypes groups studied of N0.7196 T>G.

No. 7196 T>G Genotypes	OR	Preventive Fraction / or Etiological Fraction	Fisher's probability	CI 95 %
TT	0.52	0.347	0.286 NS	0.18- 1.53
TG	1.93	0.206	0.286 NS	0.65- 5.68
Allele distribution				
T	0.60	0.347	0.346 NS	0.23- 1.58
G	2.05	0.128	0.231 NS	0.77- 5.46

NS: Non-significant. OR: Odd Ratio. CI: confidence interval.



A **B**

Figure 4. DNA sequencing chromatogram which shows the No.7196 T>G/ A: the arrow points to the common genotype TT/ B: the arrow points to the substitution nucleotide T/G.

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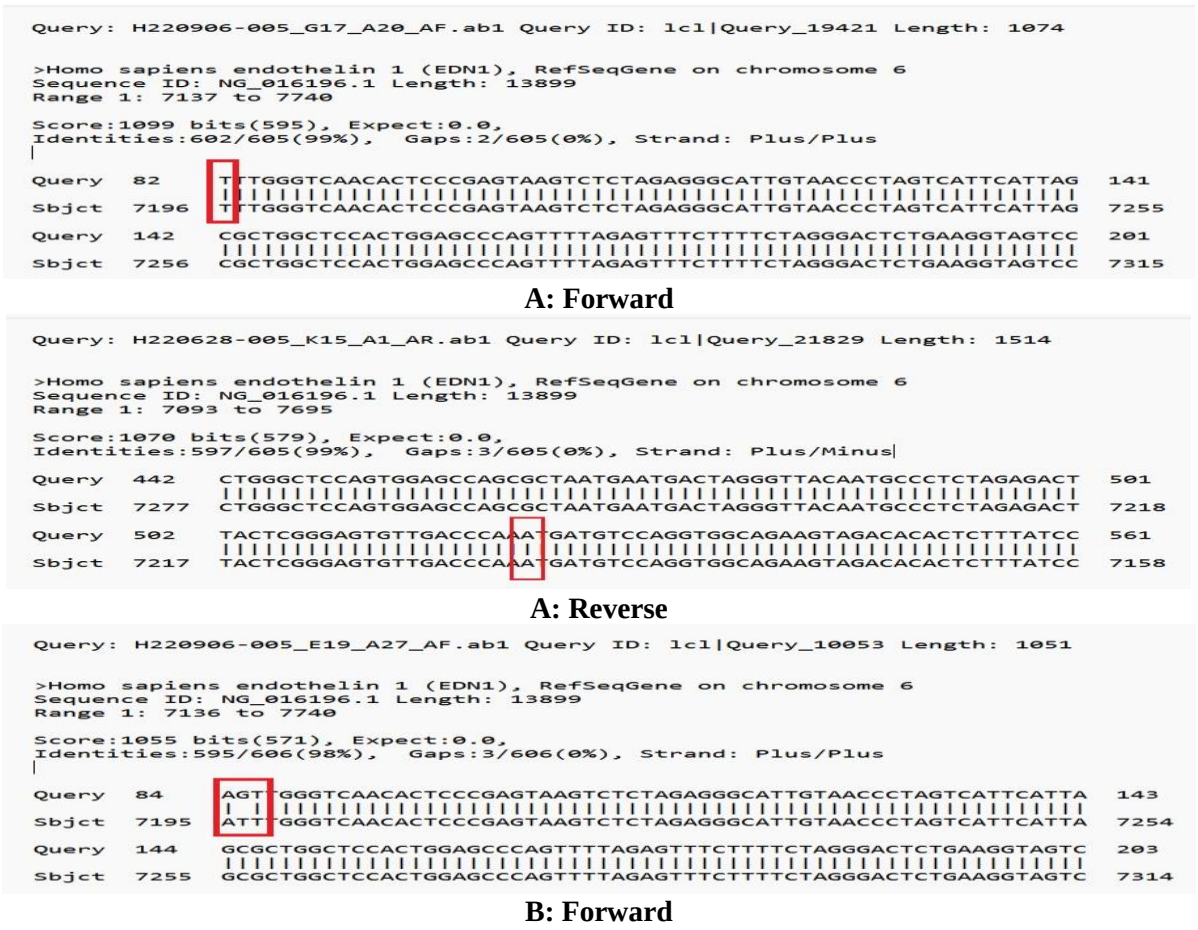

Figure 5. DNA sequence alignment on NCBI blast which shows the No.7196 T>G located on the exon 2 of the EDN1 gene chr6:12293977. A: points to the common nucleotide T in the forward and A in the reverse/ B: the arrow points to the substitution nucleotide T/G in the forward.

EDN-1 gene expression

The gene expression of EDN1 and fold change were quantified by measuring the threshold cycle (Ct) for two groups of women under the IVF program and using reference gene JHY to normalize the mRNA levels (Fig. 6). The results, as seen in Table 9, indicate that the mean Ct of the EDN1 gene was 25.53, 25.44 for the failure and success groups, respectively. While the mean of the JHY gene was 26.29 for the failure group, and 26.62 for the success group. A comparison of results reveals that the fold change in gene expression for the failure group (0.845±0.29) was lower than the fold change in the success group (1.0 ±0.00) on the day of embryo transfer, this result showed no significant differences between groups.

Table 9. The fold change of EDN-1 gene expression depends on the calculation 2^-ΔΔCt.

Study groups according to Implantation	Mean of EDN-1 Ct	Mean of JHY Ct	Mean of EDN-1 ΔCt	Mean of EDN-1 ΔCt Calibration	Mean of EDN-1 ΔΔCt	Mean of EDN-1 2^-ΔΔCt	Failure / Success	A fold of gene expression
Failure	25.53	26.29	-0.758	-1.186	0.427	3.463	3.463 / 4.096	0.845 ±0.29
Success	25.44	26.62	-1.186	-1.186	3.197	4.096	4.096 / 4.096	1.0 ±0.00
t-test	-	-	-	-	-	-	-	0.481 NS
P-value	-	-	-	-	-	-	-	0.502

NS: Non-Significant.

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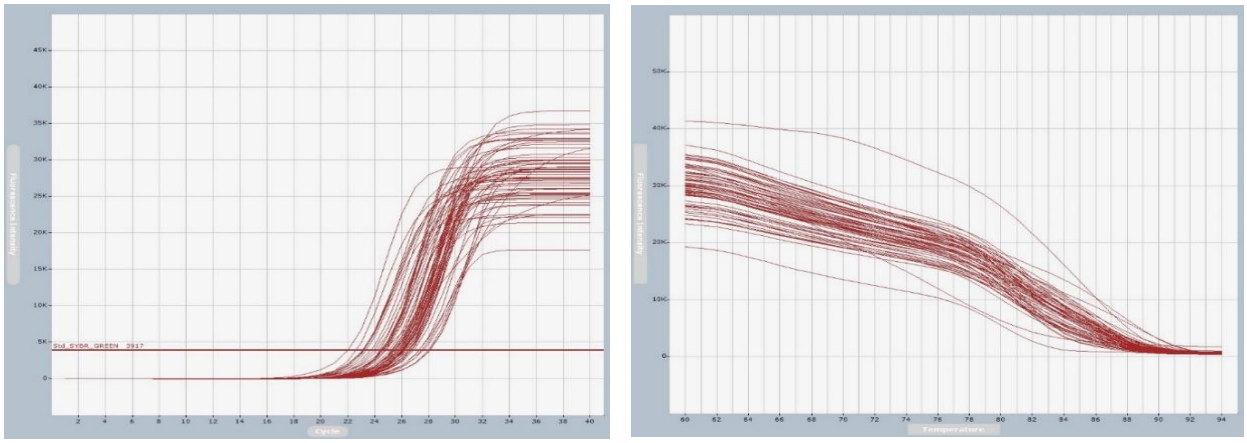


A B

Figure 6. EDN1, A: dissociation curves, B: amplification plots by qPCR Samples included both the implantation failure and implantation success study group.

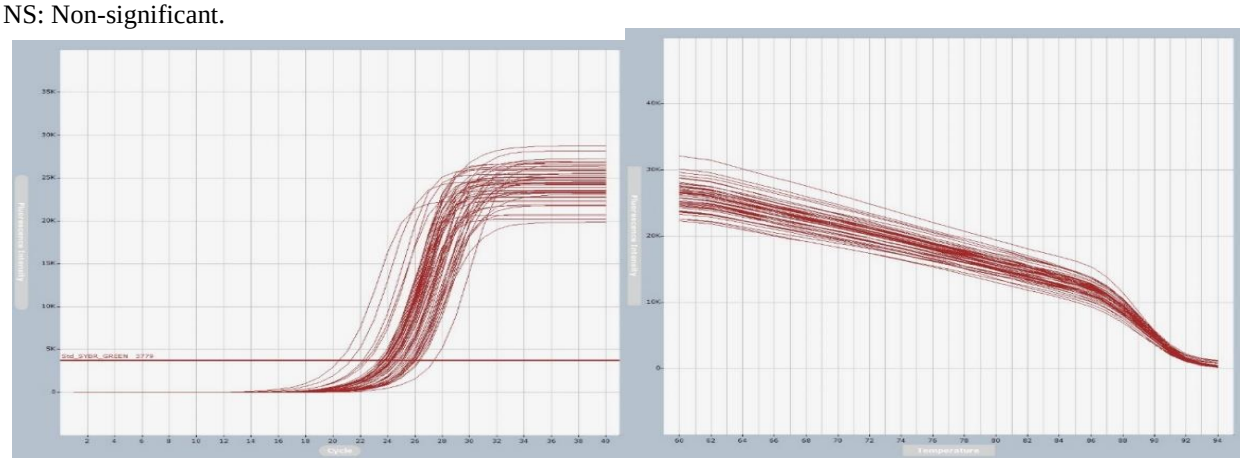
Interleukin-1β Gene

The gene expression of IL-1β and fold change were quantified by measuring the threshold cycle (Ct) for two study groups of women under the IVF program (Fig. 7), and used the reference gene JHY to normalize the mRNA level. The results, as seen in Table 10 indicate that the mean Ct of the IL-1β gene was 24.23, and 24.95 for the failure and success groups, respectively. While the mean of the JHY gene was 26.29 for the failure group and 26.62 for the success group. A comparison of results reveals that the fold change in gene expression for the failure group 0.622±0.17 was lower than, the fold change in the success group (1.0 ±0.00) on the day of embryo transfer, this result showed no significant differences between groups.

Table 10. The fold change of IL-1β gene expression depends on the calculation 2^-ΔΔCt.

Study groups according to Implantation	Mean of IL-1β Ct	Mean of JHY Ct	Mean of IL-1β ΔCt	Mean of IL-1β ΔCt Calibration	Mean of IL-1β ΔΔCt	Mean of IL-1β 2^-ΔΔCt	Failure / Success	Fold of gene expression
Failure	24.23	26.29	-2.06	-1.68	-0.38	3.203	3.203 / 5.144	0.622 ±0.17
Success	24.95	26.62	-1.68	-1.68	0.0024	5.144	5.144 / 5.144	1.0 ±0.00
t-test	-	-	-	-	-	-	-	0.407 NS
P-value	-	-	-	-	-	-	-	0.093

NS: Non-significant.



A B

Figure 7. IL-1β, A: dissociation curves, B: amplification plots by qPCR Samples included both the implantation failure and implantation success study group.

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Association of variations with gene expression

rs2070699

A study of the association between EDN1 gene variants with the gene expression as seen in Table 11 found no effect of SNP rs2070699 on the gene expression of EDN1 and IL-1β. EDN1 was higher in the GG and TT genotypes compared to the GT genotype, furthermore, their differences were nonsignificant and had mild effects.

No.7196 G>T

A study of the association between EDN1 gene variants with the gene expression as seen in Table 12 found no effect of variant No.7196 G>T in the exon 2 on the gene expression of EDN1 and IL-1β. EDN1 was higher in the TG genotype which was recorded as a risk factor compared to the TT genotype and their differences were non-significant and poor or weak effects.

Table 11. Association of variant SNP rs2070699 with study parameters.

Parameters	Mean ± SE			P-value
	GG	GT	TT	
EDN1	4.3047± 2.15878	1.3031± .47936	4.2419± 2.00062	0.638 NS
IL-1β	5.4131± 3.38511	3.1231± 1.01193	3.1496± .80615	0.721 NS

NS: Non-Significant.

Table 12. Association of variant No.7196 G>T with study parameters.

Parameters	Mean ± SE		p-value
	TT	TG	
EDN1	2.081± 0.785	6.569±2.911	0.070 NS
IL-1β	2.573±0.474	6.497±03.565	0.162 NS

NS: Non-Significant.

Correlation between study variables

The Person correlation test between EDN1, IL-1β gene expression in the current study as seen in Table 13 showed a significant positive correlation between EDN1 and IL-1β genes $P \leq 0.01$ and $P \leq 0.05$ in the failure and success groups, respectively.

Table 13. Pearson correlation between EDN1 and IL-1B gene expression in the study groups.

Groups	Parameters	EDN1	IL-1B
Failure	EDN1	-	0.760**
Implantation	IL-1B	0.760**	-
Success	EDN1	-	0.474*
Implantation	IL-1B	0.474*	-

**. Correlation is significant at P≤0.01. *. Correlation is significant at P≤0.05.

The results of the rs2090699 G>T showed no association with the embryo implantation outcome. This result also shows the homozygous genotypes GG and TT were higher frequency than the heterozygous genotype GT in both study groups, while the frequency of the T and G alleles was almost close. Even though the probability according to Fisher not significant, the OR of the GG genotype and G allele indicate that the G allele is a risk allele that may be related to infertility. Previous local studies indicated that the presence of genetic variations affects the outcome of IVF, which involves either change to implantation events or to the vascular supply throughout the early stages of pregnancy. This could cause a decrease in the success rate of the IVF program in infertile women under IVF when compared to fertile women [8-10]. We need to study many factors related to the IVF process, especially endothelin, due to its relationship to the angiogenesis and contraction of the uterine muscles. Therefore, women with the T allele are more susceptible to successful IVF treatment, while women who carry the G allele are more susceptible to failing IVF treatment.

The study recorded a new variant No.7196/ T>G, it also, shows no association with embryo implantation. The homozygous genotype TT recorded a higher frequency in the women who had successful embryo implantation. Homozygous TT had a preventive fraction effect with the common T allele of infertility. Although the heterozygous TG recorded a higher frequency in the failed females to get pregnant. The TG genotype recorded an etiological fraction effect with the G allele which may have more risk to failure IVF treatment compared to another genotype. Meanwhile, the other homozygote genotype GG was absent may be due to a small sample size and it is expected according to H.W.E by a small percentage. The sample size was limited to around 60 individuals therefore the results may occur as non-significant, In the future the research needs to increase the sample size, in order to explore the role of this Locus.

The current study describes for the first time the EDN1 gene expression in Iraqi infertile women participating in IVF programs on the day of embryo transfer. This study result shows down-regulated EDN1 gene expression in the non-pregnant women

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when compared with another group (that succeeded in implanting the embryo and became pregnant). The results are in line with Mastrogiannis and his colleagues, who found that pregnant women's plasma levels of EDN1 were higher than those of non-pregnant women but that this difference was not statistically significant[30]. There were non-significant differences, which may be due to the time of taking the samples on the day of embryo transfer, where the hormone did not reach peak expression. The level of EDN1 expression is increased at the time of blastocyst expansion, also before, and at the implantation period in the ewe uterine lumen[16]. This nonsignificant result between failure and success groups may be associated with a high level of E2 on the day of embryo transfer which results from the stimulation in the IVF program. As mentioned by Merviel and his colleagues, EDN synthesis is regulated by E2[31], also a high level of E2 on the day of transfer affects the embryo implantation [8], which may affect other factors, including EDN1 expression in women undergoing IVF.

The result of IL-1β gene expression revealed non-significant down-regulation in women who did not get pregnant, while non-significant up-regulation in the successful implantation women. IL-1β involved in communication between blastocyst and endometriosis is an important mediator of a healthy pregnancy. This result is consistent with the result of Yang and his team, which indicated no significant differences in IL-1β mRNA expression in primary ovarian insufficiency (POI) patients and healthy women who underwent IVF in the Chinese population [32]. A study by Zhu with his colleague (2019), mentions an increase in IL-1β concentrations in Chinese patients undergoing the ovarian stimulation cycle [33]. The disturbance of the proinflammatory cytokine IFN-γ, TNF-α, IL-1β, IL-6, and anti-inflammatory cytokine IL-4, IL-10, and TGF-β1 balance in peripheral blood was probably associated with recurrent implantation failure (RIF) in women following two to six IVF/ICSI–ET cycles[34]. IL-1β and TNF-a may serve as an indicator of endometrial receptivity[35]. The significant positive correlation between EDN1 and IL-1β in both study groups is consistent with the previously mentioned study, which indicates the direct relationship between EDN1 and IL-1β, IL-1 mRNA, and protein levels were enhanced by EDN1[36]. Nitric Oxide and EDN1 control the functions of endometrium in close association with IL-1β[37]. The current study concluded a finding that IVF outcome is related to genetic variation and gene expression for several factors such as EDN1 and IL-1B, this result goes with the previous Iraqi studies about different factors that affect implantation outcomes such as LIFR, LIF, Integrin, and Mucin-1 [7-10,] therefore currents factors need more focusing to study other locus and polymorphism with IVF program.

Conclusion

Genetic variants SNP rs2070699 and No.7196 in EDN1 do not have an effect on the level of gene expression in infertility women under the IVF program. EDN1 and IL-1β gene expression have a significant positive relationship in the luteal phase and both genes are up-regulated in women with successful implantation. The current results, can emphasize the importance of studying genetic factors, such as polymorphisms and gene expression, in the success of IVF programs, as many of them can improve the IVF program and develop successful results.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

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Authors' Contribution Statement

A. M. S. A. designed the study idea, design the primers of the study, data analysis, with interpretations, proofreading, and writing. Z. J.A. collected the sample, data analysis, enrolls the results, writing, and discussion.

Journal Declaration:

The second author A. M. S. A. is an editor for the journal but did not participate in the peer review process other than as an author. The authors declare no other conflict of interest.

References

1. Daud N, Gustia K. In-Vitro Fertilizatiion (IVF) Concept According to Modern Medical. IJ-ARBSS. 2023; 13; 6: 1254-1260. http://dx.doi.org/10.6007/IJARBSS/v13-i6/17571.
2. Afnan MA, Rudin C, Conitzer V, Savulescu J, Mishra A, Liu Y, et al. Ethical implementation of artificial intelligence to select embryos in in vitro fertilization. In Proceedings of the 2021 AAAI/ACM Conference on AI, Ethics Soc. 2021; 316-326. https://doi.org/10.1145/3461702.3462589.
3. Karizbodagh MP, Rashidi B, Sahebkar A, Masoudifar A, Mirzaei H. Implantation window and angiogenesis. J Cell Biochem. 2017; 118(12): 4141-51. https://doi.org/10.1002/jcb.26088.
4. Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. Am J Anat. 1956; 98(3): 435-93. https://doi.org/10.1002/aja.1000980306.
5. Maia Bezerra NK, de Menezes Galvao AC, Martins Leite NE, Leão Barbalho Sant'anna A, de Medeiros Garcia Torres M, Galvao Pinto Coelho MC, et al. Success of in vitro fertilization and its association with the levels of psychophysiological stress before and during the treatment. Health Care Women Int. 2021; 42(4-6): 420-45. https://doi.org/10.1080/07399332.2020.1787415.
6. Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. J Endocrinol. 2011; 210(1): 5-14. https://doi.org/10.1530/joe-10-0461.
7. Hassab ZS, Almohaidi AMS, Alsaidi FS. Association between the Genetic Variation of LIF R Gene with Its Gene Expression and Implantation Outcomes of IVF-Iraqi Arab Females. J Pharm Negat. 2022; 13(4): 1665- 1671. http://dx.doi.org/10.47750/pnr.2022.13.04.230
8. Yousif RA, Almohaidi AMS, Al-Musawi BJ. Assessment of ITGB3 gene expression and hormonal status in infertile female undergoing an in vitro fertilization protocol. HIV Nursing. 2023; 23(1): 770-8.
9. Yousif RA, Almohaidi AM. Identification of ITGB3 Polymorphisms with implantation outcome during in vitro fertilization program. HIV Nursing. 2023; 23(1): 257-64. https://doi.org/10.31838/hiv23.01.42.
10. Mirza SF, Saeed RH, Almohaidi AMS, Al-Janabi IJ. Evaluation mucin 1 polymorphism and expression with infertility in Iraqi females. Res Mil. 2022; 14; 12(2): 6916-27.
11. Mihanfar A, Sadigh AR, Fattahi A, Latifi Z, Hasanzadeh-Moghadam M, Samadi M, et al. Endothelins and their receptors in embryo implantation. J Cell Biochem. 2019; 120(9): 14274-84. https://doi.org/10.1002/jcb.28983.
12. Mubarak HJ, Ahmed SA. Histomorphometric and endothelin-1 immunohistochemical study of human placental villi correlated with umbilical cord coiling index. Iraqi JMS. 2017; 15 (2): 198-205.
13. Khidhir SM, Mahmud AM, Maulood IM. Association of Endothelin-I and A symmetric Dimethylarginine Levels with Insulin Resistance in Type-2 Diabetes Mellitus Patients. Baghdad Sci J. 2022; 19(1): 0055. http://dx.doi.org/10.21123/bsj.2022.19.1.0055.
14. Farhan TM. Endothelin-1 Expression in Placental Tissue Vasculature in Normal Vaginal Delivery and Cesarean Sections. Muthanna Med J. 2019; 6(1): 26-36.
15. Arinami T, Ishikawa M, Inoue A, Yanagisawa M, Masaki T, Yoshida MC, et al. 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. Am J Hum Genet. 1991; 48(5): 990.
16. *National Center for Biotechnology Information*. National Library of Medicine. EDN1 endothelin 1 - *Homo sapiens* (human).. (Accessed: 01 March 2023).
17. Mashkina EV, Kovalenko KA, Miktadova AV, Shkurat MA. Association of Gene Polymorphisms of Antioxidants with Reproductive Losses. Russ J Genet. 2020; 56: 354-62. https://doi.org/10.1186/1741-7015-11-154.
18. Flores JA. Gene expression of endothelin-1 in the porcine ovary: follicular development. Biol Reprod.

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https://doi.org/10.21123/bsj.2024.9275
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Baghdad Science Journal

2000; 63(5): 1377-82. https://doi.org/10.1093/biolreprod/63.5.1377.
19. Apa R, Miceli F, Feo DD, Pierro E, Ayala G, Mancuso S, et al. Endothelin-1: expression and role in human corpus luteum. Am J Reprod Immunol. 1998; 40(5): 370-6. https://doi.org/10.1111/j.1600-0897.1998.tb00068.x.
20. Riley SC, Findlay JK, Salamonsen LA. Endothelin-1 and endothelin receptors are present in the sheep uterus and conceptus at implantation. J Endocrinol. 1995; 147(2): 235-44. https://doi.org/10.1677/joe.0.1470235.
21. Inoue A, Yanagisawa M, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T. The human preproendothelin-1 gene: complete nucleotide sequence and regulation of expression. J Biol Chem. 1989; 264(25): 14954-9. https://doi.org/10.1016/S0021-9258(18)63795-0.
22. Bulun SE. Steroids, cytokines, and implantation. Endocrinology. 2017; 158(6): 1575-6. https://doi.org/10.1210/en.2017-00407.
23. Telford JL, Macchia G, Massone A, Carinci V, Palla E, Melli M. The murine interleukin 1β gene: structure and evolution. Nucleic Acids Res. 1986; 14(24): 9955-63. https://doi.org/10.1093/nar/14.24.9955.
24. *National Center for Biotechnology Information.* National Library of Medicine. IL1B interleukin 1 beta- *Homo sapiens* (human). (Accessed: 01 March 2023).
25. Fontana VA, Sanchez M, Cebral E, Calvo JC. Interleukin-1β regulates metalloproteinase activity and leptin secretion in a cytotrophoblast model. Biocell. 2010 34(1): 37-43. https://doi.org/10.32604/BIOCELL.2010.34.037
26. Rébé C, Ghiringhelli F. Interleukin-1β and cancer. Cancers. 2020; 12(7): 1791. https://doi.org/10.3390/cancers12071791.
27. Sharma S, Godbole G, Modi D. Decidual control of trophoblast invasion. Am J Reprod Immunol. 2016; 75(3): 34150. https://doi.org/10.1111/aji.12466.
28. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protoc. 2008; 3(6): 1101-8. https://doi.org/10.1038/nprot.2008.73
29. Bińkowski J, Miks S. Gene-Calc [Computer software]. 2018. may, 2023.
30. Mastrogiannis DS, O'Brien WF, Krammer J, Benoit R. Potential role of endothelin-1 in normal and hypertensive pregnancies. J Obstet Gynaecol Res. 1991; 165(6) :1711-6. https://doi.org/10.1016/0002-9378(91)90020-R
31. Merviel P, Lourdel E, Cabry R, Boulard V, Brzakowski M, Demailly P, et al. Physiopathology of human embryonic implantation: clinical incidences. Folia Histochem. Cytobiol. 2009; 47(5): 25-34. https://doi.org/10.2478/v10042-009-0058-3
32. Yang H, Pang H, Miao C. Ovarian IL-1α and IL-1β levels are associated with primary ovarian insufficiency. Int J Clin Exp Path. 2018; 11(9): 4711.
33. Zhu X, Niu Z, Ye Y, Xia L, Chen Q, Feng Y. Endometrium cytokine profiles are altered following ovarian stimulation but almost not in subsequent hormone replacement cycles. Cytokine. 2019; 114: 6-10. https://doi.org/10.1016/j.cyto.2018.11.002.
34. Liang PY, Diao LH, Huang CY, Lian RC, Chen X, Li GG, et al. The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. Reprod Biomed Online. 2015; 31(6): 823-6. https://doi.org/10.1016/j.rbmo.2015.08.009.
35. Boomsma CM, Kavelaars A, Eijkemans MJ, Lentjes EG, Fauser BC, Heijnen CJ, et al. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum Reprod. 2009; 24(6): 1427-35. https://doi.org/10.1093/humrep/dep011.
36. Rikimaru T, Awano S, Mineoka T, Yoshida A, Ansai T, Takehara T. Relationship between endothelin-1 and interleukin-1β in inflamed periodontal tissues. Biomed Res. 2009; 30(6): 349-55. https://doi.org/10.2220/biomedres.30.349.
37. Lin Z, Kubota T, Masuda M, Aso T. Role of nitric oxide synthase in release of endothelin from cultured human endometrial cells. Eur J Endocrinol. 1998; 138(4): 467-71. https://doi.org/10.1530/eje.0.1380467.

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تعدد الاشكال لجين الاندوثيلين-1 مع التعبير الجيني للبين ابيضاضي-1 بيتا في النساء العراقيات المصابات بالعقم الخاضعات لبرنامج الاخصاب خارج الرحم

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الخلاصة

تقبل بطانة الرحم هي المرحلة المحددة لنجاح العلاج ببرنامج أطفال الانابيب، يتم تنظيم غزو خلايا الأرومة الغاذية البشرية لبطانة الرحم من خلال العديد من العوامل مثل EDN1 و IL-1β. تهدف هذه الدراسة الى التحقق من العلاقة بين تعدد الاشكال والتعبير الجيني لجينات EDN1 و IL-1β وانغراس الجنين في النساء المصابات بالعقم الخاضعات لبرنامج الاخصاب خارج الرحم. تم جمع عينات الدم المحيطي في يوم الارجاع بعد ساعة من ارجاع الجنين. بعد اخذ الموافقة من 60 امرأة وتم تقسيمها الى مجموعتين وفقا لنتيجة انغراس الجنين، مجموعة فشل لديهن الانغراس شملت 35 امرأة، ومجموعة نجح لديهن الانغراس شملت 25 امرأة. كشفت نتائج تتابع تسلسل الحمض النووي عن وجود تغايرين وراثيين في جين EDN1، rs2070699 لم يظهر أي ارتباط بنتيجة انغراس الجنين في النساء المصابات بالعقم وفقا لاحتمالية فيشر، واظهر تواترا عاليا في مجموعتي الدراسة للطرز الوراثية GG و TT مقارنة بالطراز الوراثي GT. ويظهر التغاير No.7196T>G على الإكسون الثاني ان الطراز الوراثي TT كان الأعلى تكرارا في مجموعة النجاح، بينما الطراز الوراثي TG هو الأعلى تكرارا في مجموعة الفشل. نتائج التعبير الجيني لجين EDN1 و IL-1β لم تظهر أي اختلاف معنوي بين مجموعتي الدراسة، واظهر الارتباط بين EDN1 و IL-1β علاقة إيجابية معنوية. نستنتج، ان التغايرات الوراثية rs2070699 و No.7196T>G ليس لها تأثير على مستوى التعبير الجيني عند النساء المصابات بالعقم الخاضعات لبرنامج أطفال الانابيب. التعبير الجيني لجين EDN1 و IL-1β يمتلكان علاقة إيجابية مهمة في المرحلة الأصفرية وكلاهما يزداد التعبير عنه في النساء اللواتي لديهن انغراس جنيني ناجح.

الكلمات المفتاحية: جين الاندوثيلين-1، التعبير الجيني للاندوثيلين-1، جين البين ابيضاضين-1، النساء العراقيات المصابات بالعقم، برنامج الاخصاب خارج الرحم.