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Original Article

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The Association of Soluble VEGFR-1 Serum Level and Genetic (rs7993418) Polymorphism with In Vitro Fertilization and Embryo Transfer Outcome in the Population of Northern Iran

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Abstract

Background: Vascular endothelial growth factor receptors ($VEGFR_S$) play an important role in embryo implantation. The aim of the present study was to examine the association of VEGFR1 circulating level and gene polymorphism with in vitro fertilization and embryo transfer (IVF-ET) outcome.

Methods: In this case–control study, 120 women who had unsuccessful IVF (IVF^{-}) history and 120 women who had successful IVF outcome (IVF^{+}) as controls were included. Genomic DNA was extracted from blood samples. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The serum levels of soluble VEGFR1 (sVEGFR1) were measured by ELISA. ANOVA test was used for statistical analysis.

Results: The frequency of T and C alleles in IVF^{+} individuals were 87.5%, 12.5% and among IVF^{-} were 75.5%, 24.5%, respectively (p=0.0006). The minor allele (C) was associated with an increased risk of IVF failure based on results from co-dominant (OR=3.86, 95%CI 1.19-12.47), dominant (OR=2.32, 95%CI 1.31-4.10), recessive (OR=3.22, 95%CI 1.00-10.29), and allele models (OR=2.28, 95%CI 1.40-3.69). We also showed that there is a significant decrease in serum sVEGFR1 levels in IVF as compared to IVF^{+} (p=0.006) groups. Moreover, TT genotype is significantly associated with increased serum sVEGFR1 concentration in IVF group (TT, CT, and CC serum levels were 106.55±11.04, 94.33±10.75, and 83.33±9.13 *ng/ml*, and in IVF^{+} group were 156.11±18.08, 120.66±16.51, and 84.66±20.31 *ng/ml*, respectively).

Conclusion: The results of this study indicate that VEGFR1 polymorphism and sVEGFR1 circulating levels are associated with IVF-ET outcome. Moreover, CC genotype is associated with decreased sVEGFR-1 serum concentration and IVF-ET failure.

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Introduction

Infertility is a major health problem affecting about 15% of couples trying to have a child (1). Moreover, it is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Of all couples classi-

fied as infertile, female infertility accounts for about 40–50%. In 30–40% of infertile couples, male infertility is the cause, while the remaining 10–30% is attributed to both male and female infertility (2).

In vitro fertilization and embryo transfer (IVF-ET) is the most successful infertility treatment and for many infertile women is the last chance of pregnancy (3). Despite progress in assisted reproductive technology (ART), including IVF-ET, people undertaking IVF often face great risk of failure. There are many possible factors that may be responsible for this failure, including inappropriate stimulation protocol, genetic abnormality in the gametes, and embryo implantation failures (4). Embryo implantation is an important step for establishing pregnancy which requires molecular and cellular events resulting in uterine growth and differentiation, blastocyst adhesion, invasion, and placental formation. Implantation is a complex process which requires synchronization of events in the developing embryo and receptive endometrium, and involves factors such as angiogenic factors, cytokines, growth factors, and cell adhesion molecules (5).

After invading the maternal endometrium, embryonic development is characterized by a dramatic growth of vascular membranes and the formation of the placenta; this is regulated mainly by VEGF system and VEGF receptors (VEGFRs) have been shown to be expressed by endometrium during the time of implantation (6).

VEGFRs signaling in vertebrates play essential roles in the regulation of angiogenesis. The VEGFR1 gene is located on the long (q) arm of chromosome 13 at position 12.3, spans approximately 19 *Kb*, and contains 33 exons. The VEGFR-1 gene produces two major proteins: a full-length receptor and sFlt-1. Transmembrane VEGF receptors are expressed mostly on endothelial cells and hemangioblasts, but Flt-1 could be detected in human trophoblast and choriocarcinoma cells (7). VEGFR1 is structurally a typical receptor tyrosine kinase of about 180 *kDa*. Early in embryogenesis, null mutation of VEGFR1 results in lethality due to a disorganization of blood vessels and an overgrowth of endothelial-like cells, suggesting a regulatory role *in vivo* (8). Indeed, VEGFR-2 knockout mice exhibit a lethal phenotype at embryonic day 8.5 due to a lack of vasculogenesis (9). Transmembrane VEGF receptors are expressed mostly on endothelial cells and they can be detected in human trophoblast and choriocarcinoma cells (10, 11). Expression of VEGF receptors occurs in endometrium at peri-implantation stages and is involved in mediating vascular hyperpermeability necessary for blastocyst implantation (12).

Genetic polymorphism plays an important role in the IVF-ET outcome (13-15). VEGFR signaling has been shown to be important in placenta formation by increasing blood vessels formation, and one of the most important polymorphisms identified within the promoter of VEGFR1 gene is rs7993418 (with minor allele frequency of 0.27), regulating the VEGFR1 expression; accordingly, the purpose of the current study was to investigate the association of VEGFR1 (rs7993418) gene polymorphism and its serum concentration with IVF-ET outcome.

Methods

Samples: In this case–control study, 120 infertile women who had unsuccessful IVF (IVF^-) history and 120 infertile women who had successful IVF outcome (IVF^+) as control subjects were included. The control group (IVF^+) consisted of women who became pregnant with the first embryo transfer. The IVF negative group included cases who experienced three or more failed in vitro fertilization (IVF) attempts with good quality embryos. Patients were recruited from IVF section of Alzahra Hospital, Rasht, Iran. The study was conducted between October 2015 and May 2016. A structured questionnaire was used to obtain information on demographic features. The inclusion criteria referred to women having suffered two or more recurrent implantation failures (RIFs) after at least 2 consecutive cycles of IVF or ICSI in which a high-quality embryo had been transferred during each cycle. The exclusion criteria were anatomic abnormalities, maternal pathology, maternal inflammatory diseases, uterine malformation, diabetes, endocrine dysfunction, autoimmune diseases, prior chemotherapy, urogenital infection, patients with polycystic ovary syndrome (PCOS based on Rotterdam 2003 criteria), severe male infertility, and inflammatory pelvic diseases. Fallopian tube damage or blockage, ovulation disorders, endometriosis, uterine fibroids, and unexplained infertility were the main causes of referring patients to IVF section.

First, 2 *ml* of non-coagulated ethylenediaminetetraacetic acid (EDTA) samples were collected from both groups for DNA extraction. The genomic DNA was extracted from the whole blood

by using DNA extraction techniques and GPP solution kit (CinnaGen, Iran). Extracted DNA was visualized and confirmed by electrophoresis on 0.1% agarose gel. The extracted DNA samples were stored at -70°*C* until analysis. Blood samples were collected, centrifuged within the first 30 *min*, and the resulting sera were stored at -80°*C* to avoid possible changes in sVEGFR1 concentrations. All samples were evaluated in duplicate using a commercially available ELISA kit, according to the manufacturer's instructions. Informed consent was obtained from all individual participants included in the study. The study was accepted by the University of Guilan Ethics Committee (95- 8659) and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Analysis of genetic polymorphism: For genotyping VEGFR-1 polymorphism (rs7993418), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used. The PCR primers were synthesized. Primer sequences were as follows: 5'-CAGCCACTCCTGAATCC AGA -3' (forward) and 5'-CTGATTTCACAAC GGACCAG -3' (reverse). The amplification procedure was carried in a total reaction volume of 20 *µl*, containing 10 *µl* 2X PCR Master Mix (CinnaGen, Iran), 1 *µl* forward primer, 1 *µl* reverse primer, 3 *µl* sterile deionized water, and 5 *µl* template DNA. The amplification included initial denaturation at 94°*C* for 5 *min*, amplification for 35 cycles at 94°*C* for 45 *s*, 52°*C* for 45 *s* and 72°*C* for 45 *s*, followed by a final elongation step at 72°*C* for 5 *min*. The PCR products were visualized on a 2% agarose gel containing ethidium bromide under UV illumination. To confirm the accuracy of genotyping results, genotyping was randomly repeated in 10% of subjects to obtain concordance by minimizing genotyping errors. Then, the PCR products were digested for 2 *hr* at 37°*C* with 2 units of SnaBI (Thermo Scientific Eco105I), and the amplified fragment of 469 *bp* was cut into fragments of 264 and 205 *bp* and visualized on a 2% agarose gel containing ethidium bromide under UV illumination. This method is able to detect all three possible genotypes for the polymorphism including homozygous wild type (TT: 469 *bp*), heterozygous variant type (TC: 469, 264 and 205 *bp*), and homozygous variant type (CC: 264 and 205 *bp*).

Using ELISA to determine soluble VEGFR1 serum concentration: Circulating VEGFR1 levels were measured using the sensitive two site ELISA and antiserum against human VEGFR1. Human VEGFR1/Flt-1 ELISA kit (Abcam, UK) was used for measurement of VEGFR1 serum concentration, according to the manufacturer's instructions.

Statistical analysis: Statistical analysis was done using χ^2 by MedCalc software *vs*. 12.1.4 (MedCalc Software Ltd, Belgium) in order to estimate the association between polymorphism of VEGFR1 gene and IVF-ET outcome. Odds ratio was calculated using Cochran-Mantel-Haenszel test. Data were analyzed by chi square test, analysis of variance (ANOVA), and by calculating 95% confidence interval (CI). ANOVA was used to determine if there is a statistically significant difference between the groups. The p-value of less than 0.05 was considered statistically significant. Tukey test was applied for post hoc analysis.

Results

In the present study, 240 subjects including 120 IVF^- and 120 IVF^+ were recruited. The IVF failure group had a mean age of 31.15±6.12 (age range, 23–42) and in IVF positive group, the mean age was 32.45±7.41 years (age range, 22–40). No significant difference was seen in the age between the two groups (p=0.54).

The length of PCR product for VEGFR-1 was 469 *bp* (Figure 1). Genotyping of VEGFR-1 was done using PCR-RFLP method (Figure 2). All information about allele and genotype frequencies for IVF^- and IVF^+ groups is presented in table 1. During genotype analysis, post hoc analysis revealed significant differences between TT-TC ($p< 0.0001$), TT-CC ($p<0.0001$), and TC-CC ($p< 0.0001$) in both IVF^+ and IVF^- groups.

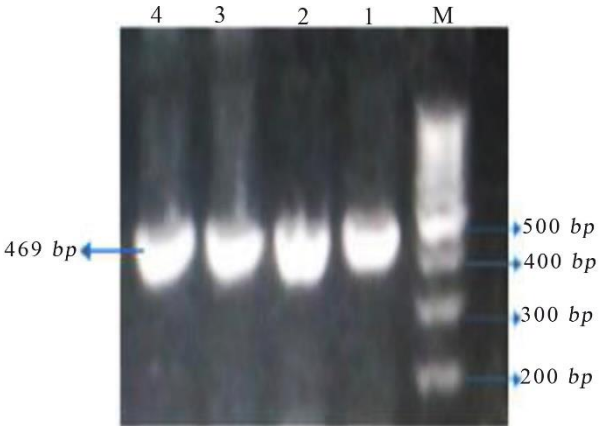

Figure 1. Agarose gel electrophoresis stained by safe stain after PCR amplification of VEGFR1. "M" represents "Marker". The size of PCR product was 469 *bp*

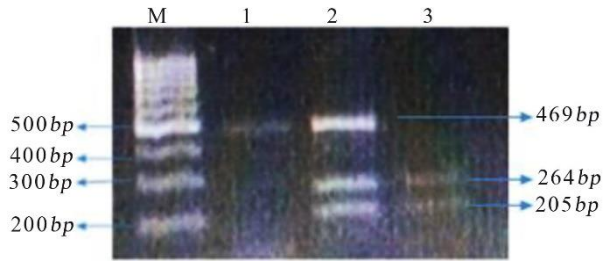

Figure 2. PCR-RFLP products. M=Molecular marker, TT= 469 *bp* (lane 1), TC=205, 264, 469 *bp* (lane 2), and CC=205 and 264 *bp* (lane 3)

The frequencies of T and C alleles in IVF$^+$ individuals were 87.5% and 12.5% and among IVF$^-$ were 75.5% and 24.5%, respectively (χ^2=11.60, p=0.0006). The minor allele (C) was associated with an increased risk of IVF failure based on results from co-dominant (OR=3.86 [95%CI 1.19-12.47] p=0.02), dominant (OR=2.32 [95%CI 1.31-4.10] p=0.003), recessive (OR=3.22, [95%CI 1.00-10.29] p=0.04), and allele models (OR=2.28 [95%CI 1.40-3.69] p=0.0008).

Using enzyme linked immunosorbent assay (ELISA), it was shown that levels of serum sVEGFR1 in the IVF$^-$ group (98.12±12.12 *pg/ml*) was significantly lower than in IVF$^+$ group (119.45±13.11 *pg/ml*) (p=0.006) (Figure 3).

It was also shown that TT genotype is significantly associated with increased serum sVEGFR1 concentration in IVF$^-$ group (TT, CT, and CC serum levels were 106.55±11.04, 94.33±10.75, and 83.33±9.13 *pg/ml*, and in IVF$^+$ were 156.11± 18.08, 120.66±16.51, and 84.66±20.31 *pg/ml*, respectively) (Figures 4 and 5).

Discussion

Infertility has become an increasingly common health problem and has been estimated to affect approximately 10% of women in the reproductive age. Since the first successful treatment with conventional in vitro fertilization (IVF) in 1978, assisted reproductive technology (ART) has become an integral part of modern medicine and now plays a key role in the fulfillment of family planning. At least five million infants have been born

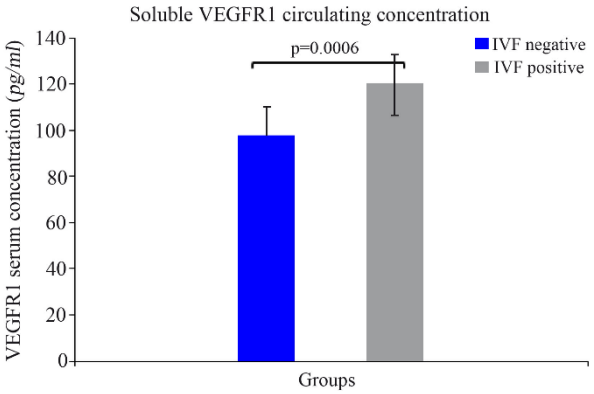

Figure 3. s-VEGFR1 level in the serum samples of IVF$^-$ and IVF$^+$ groups (*pg/ml*)

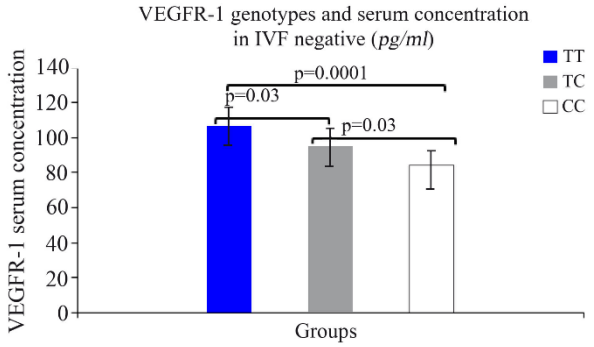

Figure 4. Association of sVEGFR1 serum concentration and genotypes in IVF$^-$ group

Table 1. Genotype frequencies of VEGF promoter polymorphism in IVF$^-$ and IVF$^+$ groups

Model	Genotype	IVF$^+$ N (%)	IVF$^-$ N (%)	OR (95%CI)	p-value
Co-dominant	TT	94 (78.33)	73 (60.83)	1.00	-
	TC	22 (18.33)	35 (29.16)	2.04 (1.10-3.78)	0.02
	CC	4 (3.33)	12 (10)	3.86 (1.19-12.47)	0.02
Dominant	TT	94 (78.33)	73 (60.83)	1.00	-
	TC+CC	26 (21.67)	47 (39.17)	2.32 (1.31-4.10)	0.003
Recessive	TT+TC	116 (96.67)	108 (90)	1.00	-
	CC	4 (3.33)	12 (10)	3.22 (1.00-10.29)	0.04
Over-dominant	TT+CC	98 (81.67)	85 (70.84)	1.00	-
	TG	22 (18.33)	35 (29.16)	1.83 (0.99-3.36)	0.05

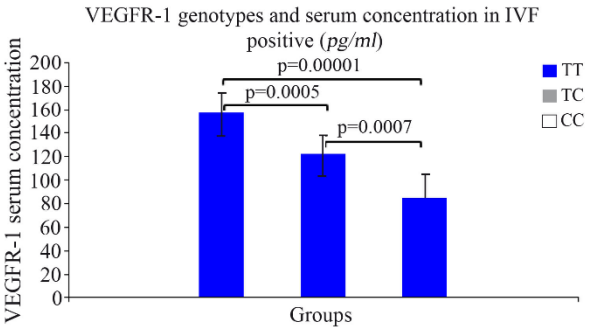

Figure 5. Association of sVEGFR1 serum concentration and genotypes in IVF^{+} group

as a result of ART and in some countries, the proportion of infants born after ART now exceeds 5% (16, 17). In general, 60% of women undergoing in vitro fertilization (IVF) treatments fail to become pregnant after the first embryo transfer and nearly 20% of patients are suffering from unexplained recurrent implantation failures (RIFs) and repeated pregnancy loss (RPL). It has been suggested that uterine receptivity is one of the important causes for implantation failure (18).

Angiogenesis, invasion, and decidualization play an important role in implantation and embryo development. Expressions of VEGF, VEGFR1, and VEGFR2 in endometrium were shown to play key role in embryo implantation (19). Increased expression of VEGFR in the endometrium, especially VEGFR-1 and -3, may be related to the receptivity of the endometrium (20).

It has been shown that change in gene expression of VEGF and its receptors in endometrium and changes of serum VEGF might play important roles in pathogenesis of unexplained recurrent spontaneous abortion (URSA) (21). Elevated maternal serum concentrations of VEGF, as early as 11 days after embryo transfer, were shown to be associated with ectopic pregnancies after IVF (22). It was shown that elevated follicular fluid VEGF concentrations are related with failure of conception in IVF cycles and may serve as markers in clinical practice (23).

It was documented that serum VEGF level is significantly lower in women with recurrent pregnancy loss compared with women with successful term pregnancy, implicating its role in maintaining pregnancy (24). It was also shown that serum sVEGF concentration in the follicular fluid decreased in patients with endometriosis (25).

Chen et al. have shown that the VEGF 936 C/T gene polymorphism may be associated to IVF-ET/ICSI treatment outcome, and C allele may be a susceptibility gene for IVF-ET/ICSI failure (26). It was shown that the VEGF-2578AA genotype, -634G allele, and -2578A/-1154A/-634G/936C haplotype may be genetic markers for susceptibility to recurrent implantation failure (RIF) (27). Jung et al. have shown that the VEGF rs833061/rs3025020 polymorphism is correlated to the development of recurrent pregnancy loss (RPL) (28). It has been shown that idiopathic recurrent spontaneous miscarriage (IRSM) frequency may depend on GC and CC genotypes of rs2010963 VEGF polymorphism (29). It has been suggested that there is significant correlation between the VEGF-1154G/A polymorphism and RIF in Brazilian women (30). In fact, VEGF-1154 G/A gene serves as a susceptibility factor affecting the chances of recurrent implantation failure (31). VEGFR1 (rs7993418) gene polymorphism was suggested to be associated with neovascular age-related macular degeneration (AMD) and metastatic clear-cell renal cell carcinoma (m-ccRCC) (32, 33). It was shown that VEGFR gene variation is associated with the risk of endometriosis and plays a crucial role in preparing the endometrium for implantation (34). Park et al. suggested that changes in VEGF concentrations in the follicular fluid are associated with poor conception rates in the IVF-ET cycles (35). It was suggested that VEGF-A concentrations were significantly elevated in women with RIF compared to normal controls (36).

In this study, there was showed that there is significant association between VEGFR1 gene polymorphism and serum concentration with IVF-ET outcome. Moreover, CC genotype is associated with decreased serum sVEGFR1 concentration and related to IVF-ET failure. The results of this research should be interpreted considering some limitations. Firstly, the size of sample has been relatively small and therefor the result should be interpreted with caution. Secondly, in this research only a population in the north of Iran has been included that may limit the application of these results to other populations. Thirdly, since only one SNP of VEGFR1 has been examined in this project, the possibility that other genetic variants could play a role in IVF-ET outcome can not be excluded. Finally, many factors act individually and together to influence IVF-ET outcome. Therefore, more factors should be included in our future research.

Conclusion

It is thus concluded that VEGFR1 circulating levels and genetic polymorphism are related with IVF-ET outcome in our population. We also suggest that CC genotype is associated with decreased serum sVEGFR-1 concentration and may be regarded as a risk factor for IVF-ET outcome. However, in order to obtain a final conclusion and confirm the results, larger studies with more samples are needed. Investigation of the relation between the frequency of SNPs in VEGFR-1 and regulation of its expression, and detailed molecular studies for the role of top-candidate genes involved in IVF-ET outcome are highly suggested for future research.

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Conflict of Interest

The authors report no conflict of interest.

References

1. Plaseska-Karanfilska D, Noveski P, Plaseski T, Maleva I, Madjunkova S, Moneva Z. Genetic causes of male infertility. Balkan J Med Genet. 2012;15 (Suppl):31-4.
2. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International committee for monitoring assisted reproductive technology (ICMART) and the World health organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009;92(5):1520-4.
3. Wright VC, Chang J, Jeng G, Chen M, Macaluso M, Centers for Disease Control and Prevention. Assisted reproductive technology surveillance-United States, 2004. MMWR Surveill Summ. 2007;56(6):1-22.
4. Mahajan N, Kaur S, Alonso MR. Window of implantation is significantly displaced in patients with adenomyosis with previous implantation failure as determined by endometrial receptivity assay. J Hum Reprod Sci. 2018;11(4):353-8.
5. van Mourik MS, Macklon NS, Heijnen CJ. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol. 2009;85(1):4-9.
6. Lai TH, Vlahos N, Shih IeM, Zhao Y. Expression patterns of VEGF and Flk-1 in human endometrium during the menstrual cycle. J Reprod Infertil. 2015; 16(1):3-9.
7. Cottrell HN, Wu J, Rimawi BH, Duran JM, Spencer JB, Sidell N, et al. Human endometrial stromal cell plasticity: Reversible sFlt1 expression negatively coincides with decidualization. Hypertens Pregnancy. 2017;36(2):204-11.
8. Shibuya M. Structure and dual function of vascular endothelial growth factor receptor-1 (Flt-1). Int J Biochem Cell Biol. 2001;33(4):409-20.
9. Xiong Y, Huo Y, Chen C, Zeng H, Lu X, Wei C, et al. Vascular endothelial growth factor (VEGF) receptor- tyrosine 1175 signaling controls VEGF-induced von Willebrand factor release from endothelial cells via phospholipase C-gamma 1- and protein kinase A-dependent pathways. J Biol Chem. 2009;284(35):23217-24.
10. Millauer B, Wizigmann-Voos S, Schnürch H, Martinez R, Møller NP, Risau W, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell. 1993;72(6):835-46.
11. Clark DE, Smith SK, Sharkey AM, Charnock-Jones DS. Localization of VEGF and expression of its receptors flt and KDR in human placenta throughout pregnancy. Hum Reprod. 1996;11(5): 1090-8.
12. Halder JB, Zhao X, Soker S, Paria BC, Klagsbrun M, Das SK, et al. Differential expression of VEGF isoforms and VEGF (164)-specific receptor neuropilin-1 in the mouse uterus suggests a role for VEGF (164) in vascular permeability and angiogenesis during implantation. Genesis. 2000;26(3): 213-24.
13. Karimlo FK, Mashayekhi F, Sorouri ZZ, Bahador MH, Salehi Z. Association of GSTM1 and GSTT1 gene polymorphisms and in-vitro fertilisation outcome in a population in northern Iran. J Obstet Gynaecol. 2015;35(1):46-8.
14. Alipour M, Mashayekhi F, Salehi Z. Association of leukemia inhibitory factor gene polymorphism and in vitro fertilization outcome in a population in northern Iran. Cell Mol Biol (Noisy-le-grand). 2017;63(3):58-61.
15. Shabanipour S, Mashayekhi F, Bahadori MH, Soruri ZZ. The relationship between MMP-9 promoterpolymorphism and IVF outcome. Cell Mol Biol (Noisy-le-grand). 2015;61(1):64-7.
16. De Geyter C. Assisted reproductive technology: Impact on society and need for surveillance. Best Pract Res Clin Endocrinol Metab. 2019;33(1):3-8.
17. Crawford G, Ray A, Gudi A, Shah A, Homburg R. The role of seminal plasma for improved outcomes during in vitro fertilization treatment: review of the

literature and meta-analysis. Hum Reprod Update. 2015;21(2):275-84.

18. Goodman C, Jeyendran RS, Coulam CB. Vascular endothelial growth factor gene polymorphism and implantation failure. Reprod Biomed Online. 2008; 16(5):720-3.
19. Zarezade N, Saboori Darabi S, Ramezanali F, Amirchaghmaghi E, Khalili G, Moini A, et al. mRNA expression of VEGF and its receptors in fallopian tubes of women with ectopic pregnancies. Int J Fertil Steril. 2015;9(1):55-64.
20. Kim CH, Ahn JW, Kim SH, Chae HD, Kang BM. Effects on in vitro fertilization-embryo transfer outcomes of vascular endothelial growth factor receptor-1, -2 and -3 in eutopic endometrial tissue of women with endometriosis. J Obstet Gynaecol Res. 2011;37(11):1631-7.
21. Amirchaghmaghi E, Rezaei A, Moini A, Roghaei MA, Hafezi M, Aflatoonian R. Gene expression analysis of VEGF and its receptors and assessment of its serum level in unexplained recurrent spontaneous abortion. Cell J. 2015;16(4):538-45.
22. Fasouliotis SJ, Spandorfer SD, Witkin SS, Liu HC, Roberts JE, Rosenwaks Z. Maternal serum vascular endothelial growth factor levels in early ectopic and intrauterine pregnancies after in vitro fertilization treatment. Fertil Steril. 2004;82(2):309-13.
23. Asimakopoulos B, Nikolettos N, Papachristou DN, Simopoulou M, Al-Hasani S, Diedrich K. Follicular fluid levels of vascular endothelial growth factor and leptin are associated with pregnancy outcome of normal women participating in intracytoplasmic sperm injection cycles. Physiol Res. 2005;54(3):263-70.
24. Gupta P, Deo S, Jaiswar SP, Sankhwar PL. Case control study to compare serum vascular endothelial growth factor (VEGF) level in women with recurrent pregnancy loss (RPL) compared to women with term pregnancy. J Obstet Gynaecol India. 2019;69(Suppl 2):95-102.
25. Garrido N, Navarro J, Remohí J, Simón C, Pellicer A. Follicular hormonal environment and embryo quality in women with endometriosis. Hum Reprod Update. 2000;6(1):67-74.
26. Chen H, Zheng JB, Wang DM, Xing H, Wang H. Association between vascular endothelial growth factor and clinical outcomes of IVF-ET/ICSI. J Coll Physicians Surg Pak. 2019;29(1):19-23.
27. Boudjenah R, Molina-Gomes D, Wainer R, de Mazancourt P, Selva J, Vialard F. The vascular endothelial growth factor (VEGF) +405 G/C polymorphism and its relationship with recurrent implantation failure in women in an IVF programme with ICSI. J Assist Reprod Genet. 2012;29(12):1415-20.
28. Jung YW, Ahn EH, Kim JO, An HJ, Cho SH, Kim YR, et al. Association of genetic polymorphisms in VEGF -460, -7 and -583 and hematocrit level with the development of idiopathic recurrent pregnancy loss and a meta-analysis. J Gene Med. 2018;20(9): e3048.
29. Sajjadi MS, Ghandil P, Shahbazian N, Saberi A. Association of vascular endothelial growth factor A polymorphisms and aberrant expression of connexin 43 and VEGFA with idiopathic recurrent spontaneous miscarriage. J Obstet Gynaecol Res. 2020;46(3):369-75.
30. Vagnini LD, Nascimento AM, Canas Mdo C, Renzi A, Oliveira-Pelegrin GR, Petersen CG, et al. The relationship between vascular endothelial growth factor 1154G/A polymorphism and recurrent implantation failure. Med Princ Pract. 2015;24(6): 533-7.
31. Goodman C, Jeyendran RS, Coulam CB. Vascular endothelial growth factor gene polymorphism and implantation failure. Reprod Biomed Online. 2008; 16(5):720-3.
32. Cobos E, Recalde S, Anter J, Hernandez-Sanchez M, Barreales C, Olavarrieta L, et al. Association between CFH, CFB, ARMS2, SERPINF1, VEGFR1 and VEGF polymorphisms and anatomical and functional response to ranibizumab treatment in neovascular age-related macular degeneration. Acta Ophthalmol. 2018;96(2):e201-12.
33. Beuselinck B, Jean-Baptiste J, Schöffski P, Couchy G, Meiller C, Rolland F, et al. Validation of VEGFR1 rs9582036 as predictive biomarker in metastatic clear-cell renal cell carcinoma patients treated with sunitinib. BJU Int. 2016;118(6):890-901.
34. Cardoso JV, Abrão MS, Vianna-Jorge R, Ferrari R, Berardo PT, Machado DE, et al. Combined effect of vascular endothelial growth factor and its receptor polymorphisms in endometriosis: a case-control study. Eur J Obstet Gynecol Reprod Biol. 2017;209:25-33.
35. Park SJ, Ki KD, Lee HW, Lee YB, Lee KS, Huh CY, et al. The follicular fluid and serum concentrations of vascular endothelial growth factor in patients during IVF-ET cycles. Korean J Obstetr Gynecol. 2006;49:1941-8.
36. Bansal R, Ford B, Bhaskaran S, Thum M, Bansal A. Elevated levels of serum vascular endothelial growth factor-A are not related to NK cell parameters in recurrent IVF failure. J Reprod Infertil. 2017;18(3):280-7.