

## Polymorphism of Vascular Endothelial Growth Factor Gene–1154 G/A in Women with Recurrent Miscarriage and IVF Failure; A case–control study

Maryam Eftekhari, M.D. <sup>1</sup>, Saeedeh Soleimanian, M.Sc. <sup>2</sup>, Soheila Pourmasumi, Ph.D. <sup>3</sup>, Nasrin Ghasemi, M.D. <sup>4</sup>,  
Mojgan Moshrefi, DVM. <sup>5</sup>

1- Professor, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

2- Ph.D. Candidate, Transplant Research Center Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

3- Assistant Professor, Non-Communicable Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran (Corresponding author; E-mail: spourmasumi@yahoo.com)

4- Professor, Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

5- Ph.D. Candidate, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Received: 15 April, 2019

Accepted: 7 March, 2020

### ARTICLE INFO

#### Article type:

Original Article

#### Keywords:

Recurrent miscarriage

IVF failures

Vascular endothelial growth factor

Polymorphism

### Abstract

**Background:** Vascular endothelial growth factor A (VEGFA) plays a key role in angiogenesis during human placenta formation and its abnormal expressions have been reported in placental tissues of women with recurrent miscarriage (RM).

**Objective:** The aim of this study was to evaluate the correlation of polymorphism of VEGF1154 G/A gene in RM and In Vitro Fertilization (IVF) failures.

**Methods:** The peripheral blood samples of women with RM, IVF failures and healthy women with live born children, as control group were collected. DNA samples were isolated and VEGF 1154G/A polymorphism was evaluated by polymerase chain reaction and restriction fragment length polymorphism. Single nucleotide polymorphism scanning was done using MnlII restriction enzymes for 1154 G/A.

**Results:** The findings of this study showed that the VEGF 1154 A/A and VEGF 1154 G/A mutation frequencies in both RM and IVF failure groups were significantly higher than those in the control group ( $p=0.005$ ). The homozygous AA mutant genotype frequency in the control group was 0, While in the RM and IVF failure groups it was 30% and 13.9%, respectively ( $p=0.005$ ). Moreover, the heterozygous AG genotype frequencies were higher in the RM (66.7%) and IVF failure (77.8%) compared to those in the control group (58.3%).

**Conclusions:** It was concluded that VEGF 1154 A/A and VEGF 1154 G/A polymorphisms were associated with both RM and IVF failures. However, their relation with IVF failures was more common than RM in A/G genotype, while in A/A, the RM was higher than IVF failure.

**Copyright:** 2020 The Author(s); Published by Kerman University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Citation:** Eftekhari M, Soleimanian S, Pourmasumi S, Ghasemi N, Moshrefi M. Polymorphism of Vascular Endothelial Growth Factor Gene–1154 G/A in Women with Recurrent Miscarriage and IVF Failure; A case–control study. *Journal of Kerman University of Medical Sciences*, 2020; 27 (2): 150-158.

### Introduction

Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy loss before the 20<sup>th</sup> week of gestation

(1). It is believed to affect 1-5% of couples worldwide. Having the history of pregnancy loss will increase the risk of miscarriage in subsequent pregnancies (2). It occurs in about 1

out of 300 couples (3). The risk of abortion increases from 12% in women without any previous history of abortion, to 25% after one abortion, 32% after 3 and 53% after 6 or more miscarriages (4). The Assisted Reproductive Technology (ARTs) hopes to get the strategies to minimize the problem (5). On the basis of a study in 2019, it was shown that about one fourth of the Iranian couples face primary infertility in some point of their life and 3.5% of them would suffer from primary infertility at any time of their life (6). In vitro fertilization (IVF) is a medical treatment for infertility which some time may face certain problems like failure of treatment cycle (7). IVF failure is being recognized increasingly as a serious concern for patients who have good quality embryos but fail to implant successfully and achieve successful delivery (8).

Parent's chromosomal abnormalities, embryo chromosomal abnormalities, and thrombotic problems, resulted from anti phospholipid antibody syndrome (APAS), are probably some major causes of RM and these disorders are responsible for less than 10 to 15 percent of RM (9). A successful pregnancy is associated with the interactions between embryo and endometrium, formation of the placenta and angiogenesis which is a critical factor in this way (10). In a study, scientists have found a number of genes, responsible for producing proteins which are necessary for implantation of the embryo in the uterus (11). Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis, a process that is vital for successful implantation and development of pregnancy (12). VEGF is a strong angiogenic factor which stimulates the proliferation of endothelial cells, exactly where the new vessels arise from existing vessels (13). Furthermore, VEGF has key functions in oocyte maturation, vascularization in decidua and improvement of embryo implantation in early conception (14).

Numerous researches have studied the impact of polymorphisms in VEGF in the field of reproductive genetics

and in a variety of phenotypes such as recurrent pregnancy loss, infertility and implantation failure (12,15). It has several single nucleotide polymorphisms (SNPs) in the promoter and five untranslated region (UTR). These SNPs are related to transcription or translation start sites. Some of these common sites of SNPs are at the following positions: -2578, -1498 -1154, and -634 (16).

Although there is a study related to the association of VEGF polymorphism and abortion, on the basis of the only meta-analysis done to date, it was shown that -1154G/A polymorphisms may not be significantly associated with the risk of repeated spontaneously abortion (17). Thus, still it is necessary to repeat this study to accurately evaluate the correlation between this gene and pregnancy loss. Due to the limitation of experimental samples in each study, various environmental factors in different geographical regions and the genetic differences between various populations are key factors, which would lead to different results. Therefore, it is necessary to repeat the studies in different populations, even though they are repetitive and lead to different results, because SNP allelic frequency is fluctuated in particular populations. On the other hand, to the best of our knowledge, there was no study carried out on the association of VEGF and miscarriage in Iran, hence, the current study was proposed.

On the basis of the only meta-analysis one to date, it has been explained that the VEGF gene -2578C/A, -1154G/A polymorphisms are not significantly associated with the risk of RM, while -634G/C and +936C/T polymorphisms are associated with the risk of RM (17). Moreover, it was shown that the rs1570360 variant of VEGF A/G 1154 is significantly relevant to recurrent spontaneous abortion risk among non-Asian women; but not Asian women (18). Therefore, still we cannot regard the association of the VEGF 1154 G/A SNP as a known fact, and it needs more evaluations to test whether or not

an association exists between RM and these SNPs. To the best of our knowledge, the present study is the first report on comparison of VEGF gene polymorphism in women with RM and IVF failure in Iran. The present study intended to examine the polymorphisms of VEGF-1154 gene and compare the presence of polymorphism VEGF- 1154 G/A among healthy individuals (women who had live births with no history of some disease), patients with RM (more than 2 times) and women who had a history of impanation failure after IVF (women who had 3 unsuccessful embryo transfer).

**Materials and Methods**

**Patients**

A case-control study was performed at Yazd Research and Clinical Center for Infertility from March 2015 to June 2018. The patients were recruited from among those who referred to infertility clinic. All patients signed an informed consent form after explanation presented by an expert person. The procedure was based on the ethical standards of clinical trial studies and the Helsinki Declaration of 1975. Furthermore, it was approved by Ethics Committee of Yazd Research and Clinical Center of Infertility. It was comprised of 192 women with reproductive age of 20 – 35 years. As the age would affect pregnancy outcome, to nullify the effect of age, it was tried to recruit the patients from the same range of age in all groups. In addition, the age equalization was confirmed by Pearson Chi- square test (P = 0.064), (Table 1).

As the samples were collected from the patients who referred to “Yazd research and clinical center for infertility”, a referral center for infertility in Iran, the patients were the candidates of the different Iranian populations. However, it was attempted to collect the samples from ethnically matched women. Data collection procedures were the same for patients and control subjects. Patients with endocrine disorders (diabetes & thyroid malfunction), liver disorders, hyperprolactinemia, anatomic abnormalities of the urogenital system, severe male factor infertility and coagulant disorders were excluded from the study. The samples consisted of two experimental groups and one control group. The first experimental group included women with at least three or more RM. After the physical examination and laboratory records, women with unexplained diagnosis and idiopathic pregnancy loss were identified and blood samples were collected to determine the changes of target gene. The second group included women with the history of at least 3 embryo implantation failure, after transfer of good quality embryos following IVF. The control group consisted of women with history of successful pregnancy, low risk of gynecological problems and no history of infertility, spontaneous abortion, preeclampsia, preterm delivery and ectopic pregnancy with at least two healthy children. All women were from the same range of age and were recruited simultaneously. Patients were bled based on the above-mentioned parameters and sample collection was easy and accessible.

**Table 1.** The mean range of women age in each group

Variables (age)	total	30-40	20-30
<b>Control group</b>	100%	60%	40%
	60	35	25
<b>Recurrent miscarriage</b>	100%	55%	45%
	60	32	28
<b>IVF failure</b>	100%	75%	25%
	72	54	18

**Genetic Study**

Gene information was as follows: Gene: VEGF, rs1570360 (homo sapiens), polymorphism: AGCCCGGGCCCGAGCCGCGTGTGGA[A/G]GGGCTG AGGCTCGCCTGTCCCCGCC, Chromosome: 6:43770093. Five ml of anticoagulated peripheral blood was collected in a

sterile tube containing 100 ml of 6% EDTA. After that, DNA samples were extracted by DNA purification Kit (AccuPrep Genomic DNA Extraction, Bioneer, Korea, K - 3032), according to the manufacture. The nucleotides changes of VEGF genotypes were verified with polymerase chain reaction followed by restriction endonuclease digestion for restriction fragment length polymorphism (PCR/RFLP) to generate a 206 bp product (restriction fragment length: 184 (187) + 22 (19) bp (A)) using the isolated genomic DNA as a template. High quality genomic DNA was used. In the present study, this concentration was 100 ng /  $\mu$ l. The parameters for the selection of tagging SNPs (tSNPs) were a minor allele frequency (MAF) of  $\geq 0.05$  and pairwise  $r^2$  of  $\geq 0.8$ . The genetic information of 1154G/A (rs1570360), with minor allele frequency (MAF) value of  $A=0.1887/945$  was from the dbSNP database of NCBI ([http://www.ncbi.nlm.nih.gov/projects/SNP/snp\\_ref.cgi?rs=1570360](http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1570360)).

### Gene amplification

PCR reaction was assembled by 5  $\mu$ l of DNA + master mix, containing 10X Reaction PCR buffer (2.5  $\mu$ l), MgCl<sub>2</sub> 25mM (0.75  $\mu$ l), dNTP 10mM (0.5  $\mu$ l), Primer forward 1  $\mu$ l (10 pmol), Primer Reverse 1  $\mu$ l (10 pmol), Taq polymerase (1 unit), and double distilled water (D.D.W) up to 25  $\mu$ l. The primers were (5'-TCCTGCTCCCTCCTCGCCAAT-3'), as forward and (5'-GGCGGGGACAGGCGAGCA-3'), as reverse. The thermocycler was adjusted with optimum thermal profile. In this way, 15 min at 94 °C was used as denaturation, followed by 35 cycles of denaturation at 94 °C for 1min, and 30 sec at 72 °C as annealing and extension for 1min at 72 °C. Final extension was accomplished at 72 °C for 10 min using the thermal cycler (MJ Mini, BIO RAD, USA). With restriction

endonuclease NlaIII, the PCR products were digested at 37 °C for 17 h. Electrophoresis (voltage: 110 for 35 min) of restriction endonuclease fragments was done on 2 % Tris-Boric acid (TBA) / EDTA agarose gel containing 1 $\mu$ g/mL ethidium bromide to visualize the probable gene bands. After electrophoreses, the gel was visualized under ultraviolet light transilluminator.

### Statistical analysis

The sample size was calculated with the equation where "interval confidence" = 95 %, "statistical power" = 80 %, gene frequency in control group = 5 %, gene frequency in experimental groups = 20 %, and least significant difference = 24.5 % and the sample volume was calculated 60 patients per each group. Nevertheless, the total number of the recruitment was 192 considering the patients removed or those refused to continue. For statistical analysis, Fisher's exact and chi-square tests with SPSS (version 16) software were applied to determine the allele frequency and the relationship between genotype polymorphism. The differences in VEGF genotype frequencies between the experimental and control groups were compared by chi square test.  $P \leq 0.05$  was accepted as significance. The odds ratios were estimated with the logistic regression model. The results were presented in percentage. Genotypes were in H-W equilibrium by using chi square test.

### Results

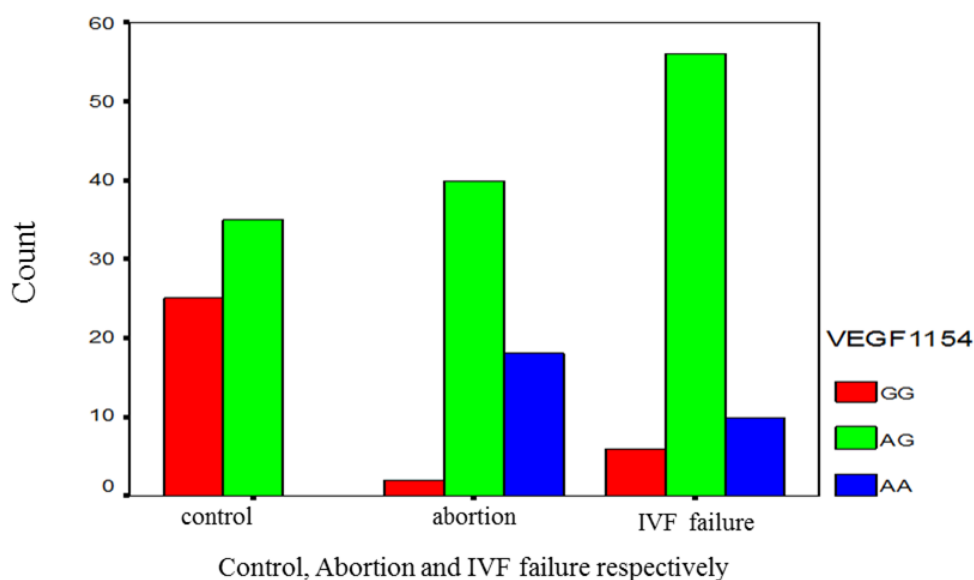
There was no significant difference between distribution and frequency of mean age in all groups.

**The genotypic frequency of polymorphism VEGF 1154 G/A**

The frequency of three genotypes: GG, AG, and AA in control group was 41.7%, 58.3%, and 0%, respectively. While in RM group, it was 3.3%, 66.7%, and 30% and in IVF failure groups it was 8.3%, 77.8%, and 13.9%, respectively. The heterozygote (VEGF 1154 G/A) and the homozygote (VEGF 1154 A/A) SNP in RM and IVF failure were higher than the control group (p=0.00). Thus, a higher frequency of AG and AA genotypes in patients (in RM and IVF failure groups) represented the inverse relationship between this single

nucleotide polymorphism (SNP) and successful embryo implantation. The frequency of homozygous (VEGF 1154 GG) genotype in the control group was 41.7, while in the RM and IVF failure groups, it was 3.3% and 8.3%, respectively (p=0.00), which showed that GG genotype frequency has positive association with successful implantation (Figure 1).

Odds ratios and 95% confidence intervals (CIs) are shown in Table 2. The Abortion and IVF failure groups showed a significantly increased risk of VEGG polymorphism compared to the control group (Table 2).



**Figure 1.** The frequency of VEGF 1154 heterozygote and homozygote genotype

**Table 2.** Odds ratio of VEGF genotype for abortion and IVF failure by logistic regression models

	VEGF1154					
	B	Odds ratio	P-Value	EXP (B)	95% C.I for EXP(B)	
<b>Abortion</b>	3.125	22.767	0.0001	22.767	5.460	94.935
<b>IVF failure</b>	2.104	8.200	0.0001	8.200	3.252	20.676

## Discussion

Our results revealed that the distribution of VEGF 1154 AG in RM (66.7%) and IVF failure (77.8%) were higher than control group (58.3%) and in IVF failure, it was higher than RM. However, VEGF 1154 AA in IVF failure (13.9%) was less than RM (30%). In control group, AA/AG genotypes, higher frequency was associated with RM and IVF failure while in healthy women with normal implantation and pregnancy, VEGF 1154 GG was higher. The genetic factors, especially chromosomal abnormalities of parents, are one of the major causes of spontaneous abortion (19). Moreover, some other factors, such as anatomical abnormalities, endocrine problems, infections and immunological factors are effective on abortion (20). On the other hand, Magnus et al. in 2019 demonstrated that the increase of delivery rate and mother age leads to increasing the risk of recurrent miscarriage (21). The human genome project leads to the increase of studies, focused on the correlation of a specific sequence of genome with a definite disease. The most common genetic markers are SNPs. Many of these variants have no effect on cell function, but according to reports, some SNPs may be related to a particular disease and help us to estimate the prevalence rate of a particular disease (22). Therefore, with the study of SNPs, we can predict the genes which are most relevant to recurrent abortion and will be able to prevent or treat the high-risk patients.

VEGF has an important role in the formation of blood vessels through the placenta development (12). The decrease of its concentration is associated with inappropriate formation of the placenta which may proceed recurrent miscarriage (23). There are some studies on VEGF-1154 polymorphisms and spontaneous abortion (24,25), however, still it is necessary to

evaluate the correlation between this gene and pregnancy loss. Several reasons support this claim including the limitation of experimental samples in each study, lack of enough knowledge about this gene, various environmental factors which are limited to each region and may influence the gene expression and the genetic differences between various populations (26). Therefore, it is necessary to repeat the studies in different populations. Maybe the studies will be repetitious but perhaps lead to different results, because SNP allelic frequency is fluctuated in particular populations. In this way, we can better clarify the relationship of VEGF gene with abortion and unsuccessful embryo implantation.

Due to the high incidence of recurrent spontaneous abortion in Iran and lack of an effective marker to screen Iranian population, it is necessary to evaluate SNPs of genes related to abortion and compare them with the results of other studies. In a recent study in India, the relationship between two SNPs of VEGF gene with recurrent pregnancy was reported. They showed that -1154G/A polymorphism increased the risk of recurrent abortion and VEGF gene, -1154 G/A and +936C/T polymorphisms were associated with RM. Papazoglou et al reported that 1154G/A genotype polymorphism is one of the reasons of idiopathic recurrent miscarriage (23). Moreover, Su et al reported that VEGF and its receptor gene (KDR) were related to idiopathic recurrent spontaneous miscarriage (27). Magdoud et al, performed a study with a large sample and reported the relation between VEGF polymorphisms, specially 1154G/A and +936C/T, with RM (28). However, in a meta-analysis in 2012, it was concluded that 1154G/A polymorphisms was not significantly associated with the risk of RM (17). Therefore, there is still controversy and more

evaluations are needed because a lot of studies after 2012 have reported the significant correlation between these two factors.

Furthermore, it was shown that VEGF polymorphism is associated with IVF failure. Shi et al., in 2017 demonstrated that there was a significant association between VEGF 1154 A/A, RM and recurrent implantation failure (29). One of the key points in the field of infertility is implantation failure after transplantation of IVF embryos (30). One of the reasons of repeated unexplained IVF failure, despite good embryo transfer, is implantation dysfunction. Sperm and oocyte quality, sperm integrity, stimulation protocol, endometrium, embryo transfer and IVF laboratory, age, BMI, obesity, uterine fibroid and anomalies, cigarette, thyroid dysfunction and immune factors are influence IVF cycle outcome (30). Previously, Boudjenah et al reported that there was a relationship between VEGF and 405 G/C polymorphism recurrent implantation failure in women under IVF cycle (31). Nevertheless, to the best of our knowledge, there is no report on the VEGF 1154 A/A polymorphism and IVF failure in a parallel study with RM.

## References

1. Dean DD, Agarwal S, Tripathi P. Connecting links between genetic factors defining ovarian reserve and recurrent miscarriages. *J Assist Reprod Genet* 2018; 35(12):2121-8.
2. Huchon C, Deffieux X, Beucher G, Capmas P, Carcopino X, Costedoat-Chalumeau N, et al. Pregnancy loss: French clinical practice guidelines. *Eur J Obstet Gynecol Reprod Biol* 2016; 201:18-26.
3. Kutteh WH. Recurrent pregnancy loss. *Obstetrics and Gynecology Clinics* 2014; 41(1):xi-xiii.
4. Tarasconi B, Tadros T, Ayoubi JM, Belloc S, de Ziegler D, Fanchin R. Serum antimullerian hormone levels are independently related to miscarriage rates after in vitro fertilization-embryo transfer. *Fertil Steril* 2017; 108(3):518-24.

## Conclusion

The results showed that SNP of homozygote (A/A) and heterozygote genotype (AG) of VEGF were related to RM and IVF failure, while homozygote genotype of G/G was associated with healthy women. Moreover, the frequency of heterozygote genotype (A/G) was higher than homozygote A/A in IVF, while the A/A frequency in RM group were higher than IVF. Therefore, it was concluded that genotype (A/G) is probably one of the most important SNPs of VEGF related to abortion. It was also concluded that VEGF 1154 A/A and VEGF 1154 G/A polymorphisms were associated with both RM and IVF failures. Nevertheless, their relation with IVF failures was more common than RM in A/G genotype, while in A/A, the RM was higher than IVF failure.

## Acknowledgment

The authors would like to thank Genetic group of Yazd Research and Clinical Center for Infertility for its financial support.

## Conflict of interest

All investigators disclose no conflict of interest in this study.



5. Alijotas-Reig J, Garrido-Gimenez C. Current concepts and new trends in the diagnosis and management of recurrent miscarriage. *Obstet Gynecol Surv* 2013; 68(6):445-66.
6. Haji Naghib Ali Hesari Z, Lotfi R, Pouragha B, Badehnoosh B, Yazdkhasti M. The need for a training software among Iranian infertile couples: a qualitative study. *Int J Fertil Steril* 2019; 13(2):118-26.
7. Patel A, Sharma PS, Kumar P. Role of mental health practitioner in infertility clinics: a review on past, present and future directions. *J Hum Reprod Sci* 2018; 11(3):219-28.
8. Koot YE, Hviid Saxtorph M, Goddijn M, de Bever S, Eijkemans MJC, Wely MV, et al. What is the prognosis for a live birth after unexplained recurrent implantation failure following IVF/ICSI? *Hum Reprod* 2019; 34(10):2044-52.
9. Dong Z, Yan J, Xu F, Yuan J, Jiang H, Wang H, et al. Genome sequencing explores complexity of chromosomal abnormalities in recurrent miscarriage. *Am J Hum Genet* 2019; 105(6):1102-11.
10. Homer H, Rice GE, Salomon C. Review: embryo- and endometrium-derived exosomes and their potential role in assisted reproductive treatments-liquid biopsies for endometrial receptivity. *Placenta* 2017; 54:89-94.
11. Kelleher AM, Peng W, Pru JK, Pru CA, DeMayo FJ, Spencer TE. Forkhead box a2 (FOXA2) is essential for uterine function and fertility. *Proc Natl Acad Sci U S A* 2017; 114(6):E1018-E26.
12. Melincovici CS, Bosca AB, Susman S, Marginean M, Miha C, Istrate M, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol* 2018; 59(2):455-67.
13. Almawi WY, Saldanha FL, Mahmood NA, Al-Zaman I, Sater MS, Mustafa FE. Relationship between VEGFA polymorphisms and serum VEGF protein levels and recurrent spontaneous miscarriage. *Hum Reprod* 2013; 28(10):2628-35.
14. Haas J, Bassil R, Gonen N, Meriano J, Jurisicova A, Casper RF. The VEGF and PEDF levels in the follicular fluid of patients co-treated with LETROZOLE and gonadotropins during the stimulation cycle. *Reprod Biol Endocrinol* 2018; 16(1):54.
15. Li SH, Hwu YM, Lu CH, Chang HH, Hsieh CE, Lee RK. VEGF and FGF2 improve revascularization, survival, and oocyte quality of cryopreserved, subcutaneously-transplanted mouse ovarian tissues. *Int J Mol Sci* 2016;17(8).
16. Sa-Nguanraksa D, O-Charoenrat P. The role of vascular endothelial growth factor a polymorphisms in breast cancer. *Int J Mol Sci* 2012; 13(11):14845-64.
17. Zhang B, Dai B, Zhang X, Wang Z. Vascular endothelial growth factor and recurrent spontaneous abortion: a meta-analysis. *Gene* 2012; 507(1):1-8.
18. Xu X, Du C, Li H, Du J, Yan X, Peng L, et al. Association of VEGF genetic polymorphisms with recurrent spontaneous abortion risk: a systematic review and meta-analysis. *PloS One* 2015; 10(4):e0123696.
19. Tur-Torres MH, Garrido-Gimenez C, Alijotas-Reig J. Genetics of recurrent miscarriage and fetal loss. *Best Pract Res Clin Obstet Gynaecol* 2017; 42:11-25.
20. Kaur R, Gupta K. Endocrine dysfunction and recurrent spontaneous abortion: an overview. *Int J Appl Basic Med Res* 2016; 6(2):79-83.



21. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Haberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *BMJ* 2019; 364:l869.
22. Maslow BS, Budinetz T, Sueldo C, Anspach E, Engmann L, Benadiva C, et al. Single-nucleotide polymorphism-microarray ploidy analysis of paraffin-embedded products of conception in recurrent pregnancy loss evaluations. *Obstet Gynecol* 2015; 126(1):175-81.
23. Aggarwal S, Parveen F, Faridi RM, Phadke S, Borkar M, Agrawal S. Vascular endothelial growth factor gene polymorphisms in North Indian patients with recurrent miscarriages. *Reprod Biomed Online* 2011; 22(1):59-64.
24. Samli H, Demir BÇ, Özgöz A, Atalay MA, Uncu G. Vascular endothelial growth factor gene 1154 G/A, 2578 C/A, 460 C/T, 936 C/T polymorphisms and association with recurrent pregnancy losses. *Genet Mol Res* 2012; 11(4):4739-45.
25. Xing X, Yan J, Zhao Y, You L, Bian Y, Chen ZJ. Association of vascular endothelial growth factor gene polymorphisms with recurrent spontaneous abortion in Chinese Han women. *Am J Reprod Immunol* 2011; 65(5):521-5.
26. Vidyadhari M, Sujatha M, Krupa P, Nallari P, Venkateshwari A. Association of genetic polymorphism of vascular endothelial growth factor in the etiology of recurrent pregnancy loss: a triad study. *J Assist Reprod Genet* 2019; 36(5):979-88.
27. Su MT, Lin SH, Chen YC. Genetic association studies of angiogenesis-and vasoconstriction-related genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Hum Reprod Update* 2011; 17(6):803-12.
28. Magdoud K, Dendana M, Herbepin V, Hizem S, Jazia KB, Messaoudi S, et al. Identification of specific vascular endothelial growth factor susceptible and protective haplotypes associated with recurrent spontaneous miscarriages. *Human Reproduction* 2012; 27(5):1536-41.
29. Shi X, Xie X, Jia Y, Li S. Maternal genetic polymorphisms and unexplained recurrent miscarriage: a systematic review and meta-analysis. *Clin Genet* 2017; 91(2):265-84.
30. Penzias AS. Recurrent IVF failure: other factors. *Fertil Steril* 2012; 97(5):1033-8.
31. 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.