© IJHG 2020 Int J Hum Genet, 20(2): 66-72 (2020)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2020/20.2.744

VEGFR2 Gene Variation is Associated with Ovarian Hyper Stimulation Syndrome

Isil Cakir[1], Ayse Ozdemir[2], Mesut Onal[2], Davut Guven[2], Sengul Tural[1*] and Idris Kocak[2]

[1]Ondokuz Mayis University Medical Faculty Department of Medical Biology, Samsun/Turkey
[2] Ondokuz Mayis University Medical Faculty Department of Gynecology and Obstetrics, Samsun/Turkey

KEYWORDS DNA Sequencing. Gene Polymorphism. Gene Variation. Ovarian Stimulation. Vascular Endothelial Growth Factor

ABSTRACT This study aimed to evaluate the effects of the Vascular Endothelial Growth Factor Receptor (*VEGFR2*) gene variant on the cases of high responses to ovarian stimulation and suffering from Ovarian Hyper Stimulation Syndrome (OHSS). This study included 57 cases (30 controls and 27 patients). Patients suffered from OHSS and controls were healthy pregnant women who had delivered children. DNA was extracted from 2 ml venous blood according to kit procedure. Polymerase Chain Reaction (PCR) was followed by next generation DNA sequencing methods performed for *VEGFR2* gene exon 7. As a result of the study, the *VEGFR2* gene rs2305948 variation was statistically significant in the patient group of women who have the high number ovum (p=0.006, χ^2= 7. 560). This variant rs2305948 may be a genetic marker for detecting women with poor and high responders before ovulation stimulation. Further and larger studies are needed in order to confirm these results.

INTRODUCTION

Today, assisted reproductive technology (ART) has become a well-established and highly efficient therapy for infertility. Previous studies have evaluated genetic polymorphisms in ovarian stimulation to explain individuals' variability in response to ovulation induction. Controlled ovarian stimulation (COS) is crucial for optimising in ART and retrieving greater numbers of high-quality oocytes. Therefore, COH plays a principal role in achieving a high ART success rate and a better outcome in terms of oocyte and embryo quality (Roque et al. 2018; Laleethambika et al. 2016).

Pharmacogenetic studies have revealed a series of genetic markers involved in COS response. While applying assisted reproductive techniques, even if the same dosage of gonadotropins is used, it may result in differences oocyte numbers and quality (Dan et al. 2015). These results negatively affect the treatment and inadequate response to gonadotropins necessitates the recurrence of IVF. In some cases, an exaggerated response to exogenous gonadotropin administration may cause Ovarian Hyperstimulation Syndrome (OHSS) that can be a life-threatening complication. The syndrome is characterised by cystic enlargement of the ovaries and a fluid shift from the intravascular to the third space due to increased capillary permeability and ovarian neoangiogenesis. Its occurrence is dependent on the administration of human chorionic gonadotrophin. The incidence of moderate OHSS is estimated to be between 3.0 and 6.0 percent, while the severe form may occur in 0.1 to 3.0 percent of all cycles (Altmäe et al. 2011). Even if the relevant hormone or intracellular messenger molecule is sufficiently present, it may not interact with the receptor due to a genetic defect in the structure of the receptor. This structural problem may cause high response or poor response to ovarian stimulation. Hence, the effect of the drug varies between individuals and the same dose of medication may cause differences in oocyte number and quality.

[*]Address for correspondence:
Sengul Tural
Associate Professor,
Ondokuz Mayis University,
Medical Faculty, Department of Medical Biology,
Samsun,Turkey
Telephone: 03623121919/2846,
E-mail: stural@omu.edu.tr

VEGF has an important role in angiogenesis but also plays an important role in embryonic development, wound healing as well as female reproductive system and configuration, formation and bone growth. Increased vascular permeability associated with VEGF has been associated with OHSS. The VEGF and its receptors also seem to play a key role in this physiological process of OHSS. Concentration of VEGF in serum and that, in OHSS patients, the amount of VEGF in the follicular fluid is frequently higher than in individuals not affected by this complication (Hanevik et al. 2012; Nouri et al. 2014; O'Brien et al. 2014). Additionally it is stated that, follicle progression can be stimulated by *VEGFR2* variants and have actions on follicular development through signal transduction pathways (Abedal-Majeh et al. 2019). The *VEGF* gene encodes one of the two receptors. This receptor, also known as kinase insert domain receptor (*KDR/VEGF2*), is a type III receptor tyrosine kinase. It functions as the main mediator of VEGF-induced endothelial proliferation, survival, migration, tubular morphogenesis and sprouting (https://www.ncbi.nlm.nih.gov/gene/3791).

Aim of the Study

Based on these findings, in this study, the researchers aimed to evaluate the effects of the *VEGF* gene variant on the patients with a high ovarian response and suffering from OHSS during assisted reproductive techniques.

MATERIAL AND METHODS

Patients

This study included 57 cases (30 controls and 27 patients) with a high number of ovum recruited from the IVF Centre at the Ondokuz Mayis University (Samsun, Turkey) between September 2015 and June 2016. Informed consent in accordance with the study protocol was approved by the ethics committee of Ondokuz Mayis University Medical Faculty (KAEK, 2015/157). A complete clinical evaluation was performed for all patients. The controls were selected by excluding the diagnosis of OHSS.

DNA Extraction

DNA was extracted from 2 ml venous blood according to kit procedure (NucleoSpin, Macherey-Nagel, Germany) and stored at -20 °C until further analysis. DNA concentration and purity were evaluated using a Jenway Genowa Nano Drop1000 Spectrophotometer (Stone Staffs, UK). All individuals signed a written consent form after being informed about the details of the study.

PCR Amplification and DNA Sequencing

The exon 7 region of the VEGFR gene was amplified with the primers F/R (F:5' -TTTCCAAGACCATAGCTTACCAT-32 ; R:5' -CAGCATCAGCATAAGAAACTTGTA-32) by using GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems, USA). The PCR was conducted in triplicate for each sample of the reaction mixture (25 μL) containing 12.5 μL of One Taq 1X Master Mix with Standard Buffer (New England Biolabs (Beijing) Ltd., China), 0.5 μLof each primers (BGI Tech Solutions Co., Ltd. (BGI-Tech), China) (0.2 μM), 2 μL of template DNA (1 ng/μL) and 9.5 μL of sterile deionised water. PCR conditions were as follows, that is, initial denaturing step at 94 °C for 3 minutes, followed by 35 cycles of 94 °C for 30 seconds, 60-62 °C for 30 seconds, 71 °C for 35 seconds, and a final extension of 7 minutes at 72 °C. Subsequently, PCR products of each sample were detected by using a 2.0 percent agarose gel and purified by using a GeneJET Gel Extraction Kit (Thermo Scientific, USA). Sequencing was performed at the Novogene Bioinformatics Technology Co. Ltd with an Illumina MiSeq platform according to protocols described by previous studies (Hanevik et al. 2012). Following the manufacturer's recommendations, a sequencing library was generated by using Nextera XT DNA Library Prep Kit for Illumina (New England Biolabs (Beijing) Ltd., China) and index codes were added (Zou et al. 2016). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, USA) and Agilent Bioanalyser 2100 system. The last step, the library was sequenced on an Illumina Miseq platform. Bioinformatics analyses. The sequences were analysed with the QIIME (Hanevik et al. 2010). The clinical diagnosis of OHSS

was made as described by Fabregues et al. in 1998, listed as over-sized connected abdominal distension, progressive increase of abdominal circumference as a result of intraperitoneal fluid accumulation, hemo-concentration that occurs as the severity of OHSS increases and oliguria due to intravascular volume reduction (Fabregues et al. 1998).

Ovulation Induction Protocol

Stimulation was performed according to the disease-fix antagonist protocol. The dosage is adjusted according to ovarian response. When the serum estrogen (E2) concentration was 40pg/ml on the 3rd day of menstruation without any visible cystic structure under ultrasound examination, induction was started at a daily dose of 225 IU of FSH. Ovarian response was followed up by transvaginal ultrasonography and E2 levels. Follicle growth was monitored by transvaginal ultrasound and 10,000 units human chorionic gonadotrophin (HCG) injection was administered when at least two or three follicles of e”18 mm were seen. Oocyte retrieval was performed 34 to 36 hours afterwards under transvaginal ultrasound by using a 17-gauge needle. A single embryo was transferred. The good quality untransferred embryos were frozen for the next transfer. Exaggerated ovarian response was defined when the number of oocytes obtained from individuals exceeded 20.

Statistical Analysis

A correlation analysis between clinical findings and genotypes was performed using the SPSS statistical program. Chi-square, probability ratios (OR) and P values were also calculated to compare allele and genotype frequencies between patient and control groups. 57 genotype analyses were performed using the SPSS program (Dean et al. 2010 Version 2.3.1). Pearson Kikare was used when the sample number was above 25, and Yates Ki Square was used when it was below 25. Fisher exact or Mid-P exact was applied when the number of samples was below 5.

The obtained results were evaluated in order to determine whether there is a relationship between polymorphic regions and infertility occurrence.

RESULTS

The present study included 57 participants and mean age of the patients was 29.59 ± 4.948 years. Minimum age of the patients was 24 years, and maximum age of the patients was 43 years. Mean of the marriage duration of the patients was 6.48 years. Mean oocyte and embryo numbers of the patients was 9.92 ± 4.481 and 18.37 ± 5.534, respectively. Hormonal laboratory findings revealed that means of FSH (mIU/ml), LH (IU/L) and E2 (ng/ml) were 6.0259 ± 1.41760, 6.4374 ± 3.76936, 38.5252 ± 18.14024, respectively (Table 1).

VEGFR2 Gene Next Generation DNA Sequencing Analysis Results

VEGFR gene rs2305948 mutant locus was found to be CC homozygote in 25 (50.0%) and CT heterozygote in 2 (28.6%) of the 27 patients studied. In the control group (n=30) CC homozygotes was detected at 25 (50.0%) and 5 (71.4%) CT heterozygotes. When genotype and allele frequencies of patients and controls were com-

Table 1: Clinical findings of patients

	Patients group (N=27)		
	Mean ± SD	*Median (Min-Max)*	*Range*
Age	29.59 ± 4.948	29.00 (24-43)	19
Marriage time (year)	6.48 ± 4.061	6.00 (2-17)	15
Hemogram	12.9625 ± 1.36535	12.9500 (9.30-15.10)	5.80
FSH (mIU/ml)	6.0259 ± 1.41760	6.10000 (4.00-9.20)	5.20
LH (IU/L)	6.4374 ± 3.76936	6.2000 (0.70-19.56)	18.86
E2 (ng/ml)	38.5252 ± 18.14024	36.9800 (3.70-99.00)	95.30
Oocyte number	18.37 ± 5.534	17.00 (13-32)	19
Embryo number	9.92 ± 4.481	10.00 (2-19)	17

pared, there was no statistically significant relationship in terms of rs2305948 region (p> 0.05). When compared to TT +CT versus CC or TT versus CC + CT from genotypes, no statistical relation was found (p> 0.05) (Table 2).

CT genotype frequency was found to be statistically significant in individuals with more than 20 oocyte counts in the study group (p = 0.006) (Table 3). In addition, no significant relationship was found between the number of patients who obtained oocytes over 15 in the patients group and the presence of CT genotype frequency (p = 0.189). Mutation-dependent genotype variation was not statistically significant with patient age (p = 0.223).

DISCUSSION

In humans, VEGF has a biological effect by binding to the VEGF1 and VEGF2 receptors. Vascular endothelial cells are also responsible for vascular permeability of VEGF (Ferrara 2004). A number of studies have examined the relationship between peripheral blood concentration of VEGF and OHSS risk. In these studies while positive associations were shown, contradictory results were found as well (Enskog et al. 2001; Pau et al. 2006).

VEGFR2 gene has a polymorphic region rs2305948 (C > T) located in exon 7 and causing amino acid change in codon 297 (valine > isoleucine). The site of amino acid substitution is in the ligand binding domain of the extracellular domain of the receptor. In vitro studies indicate that this polymorphic region affects the binding of VEGFR and VEGFR-2 kinase insert domain receptor (KDR). Clinically, it has been found that rs2305948 polymorphic region is associated with coronary artery disease, intracerebral haemorrhage and stroke (Wang et al. 2007; Zhang et al. 2009). The rs2305948 T and rs10020464-rs7692791 haplotypes have been associated with neovascular age-related macular degeneration (Fang et al. 2009). It was detected there is a polymorphism

Table 2: Distribution genotype and allele frequencies of *VEGFR* gene rs2305948 polymorphism between patients and controls

SNP	*Genotype / allele*	*Patients (n=27) (%)*	*Controls (n=30) (%)*	χ^2	*P value*	*OR (%95 CI)*
VEGFR rs2305948	CC	25(50.0)	25(50.0)	1.131	0.288	
	CT	2(28.6)	5 (71.4)			
	T T	0	0			
	CC+CT:TT	27: 0	30: 0			
	CC: TC+TT	25: 2	25: 5	1.131	0.28	2.5 (0.4428-14.11)
	C	52	55			
				0.057	0.305	2.364 (0.4392-12.72)
	T	2	5			

*Statistically significant results are written in bold.

Table 3: Genotype frequencies according to the oocyte-embryo numbers and age of patients

	Total n (%)27	*VEGFR rs2305948*			*P value*	χ^2
		CC	*CT*	*TT*		
Oocyte Number						
<20	21(77.8)	21(84)	0(0.0)	0(0.0)		
>20	6(22.2)	4(16.0)	2(100)	0(0.0)	***0.006**	7.560
Embryo Number						
<15	12(44.4)	12(48.0)	0(0.0)	0(0.0)		
>15	15(55.6)	13(52.0)	2(100)	0(0.0)	0.189	1.728
Age						
20-30	16 (59.3)	14 (56.0)	11 (0.0)	0(0.0)		
30-40	11(40.7)	11 (44.0)	0(0.0)	0 (0.0)	0.223	1.485

*Statistically significant results are written in bold.

in the promoter region of *VEGF* that affects the transcriptional activity of VEGFR (Kariyazono et al. 2004; Wang et al. 2007). However, there are also polymorphisms that affect the coding region of the *VEGF* gene and affect the binding activity to VEGF 2 of VEGF (Lledo et al. 2014).

Several genetic polymorphisms that may be effective in the development of OHSS risk have been investigated such as, *FSHR, LHB, ESR, AMHR, SHBG, CYP19, MTHFR, BMP15, GDF9, SOD2* and *p53* (Wang et al. 2007). In the present study, the frequency of CT genotype was found to be statistically significant in patients who were highly responsive to ovarian stimulation in terms of *VEGFR2* gene exon 7 rs2305948 variant region ($p = 0.006$, $\chi^2 = 7.560$). Similarly, in another study with *VEGF* gene and OHSS patients, it suggested that rs2305945 G/T polymorphic region rs2305948, rs1870378, rs2305945 (C-T-G) haplotype is effective in the development of OHSS (O'Brien et al. 2014).

In Hanevik et al.'s study, they reported that the VEGF + 405CC genotype and OHSS were statistically related (OR 3.4, 95% CI 1.01-11.7) (Hanevik et al. 2012). There was no correlation between the other 5 SNP regions and the development of OHSS. The VEGF + 405C genotype was also associated with ovarian cancer (Steffensen et al. 2010). In another study, the VEGF + 405C genotype was identified as a risk factor for the development of endometriosis (Bhanoori et al. 2005). In addition to *VEGF*, the *VEGFR* gene has also been associated with some cancers, stroke, systemic lupus erythematosus, and recurrent abortions in previous studies (McClure et al. 1994; Nouri et al. 2014; Atalay et al. 2016; Taha et al. 2016; Yueniwati et al. 2016).

Kaczemarek et al. (2007) studied the effects of *VEGF*/*VEGFR* on OHSS development, based on 116 patients with OHSS and 124 patients as the control group, the rs2071559 (VEGFR2-604), rs2305948 (VEGFR2-1192), rs1870377 (VEGFR2-1719), rs2010963 (VEGF-405), and rs111458691 (VEGFR1-519) gene regions were researched. They found that frequency of T allele VEGFR1-519 polymorphism was high in OHSS patients ($P = 0.02$, OR: 3.62, CI: 1.16-11.27) and VEGFR1-519 and VEGF-405 polymorphic regions were significantly different between patients and control group ($p = 0.02$, OR: 3.79 CI: 1.98-11.97 and $p = 0.005$ OR: 0.29, CI: 0.17 – 0.50). In this study, it was shown that VEGF and its receptor play an important role in vascular permeability and in the development of OHSS and pathophysiology of OHSS (Kaczmarek et al. 2007). In Nouri et al.'s study they found that rs2305948 (VEGFR2-1192) gene region mutations did not correlate statistically with other parameters except for the number of oocytes obtained. They also found a significant association of the VEGF405 polymorphism (rs2010963) and the VEGFR1-519 polymorphism (rs111458691) with the occurrence of OHSS (Nouri et al. 2014). In a previous study, the *FMR1* gene was analysed in primary ovarian insufficiency patients in Turkish population and 6.6 percent mutation frequency detected (Tural et al. 2015). In a recent study, Abedal-Majed et al. hypothesised that folliculat progression during puberty is affected by diet. They tested this hypothesis and reported that follicle progression can be stimulated by VEGF165 and inhibited by VEGF165b through diverse signal transduction pathways (Abedal-Majeh et al. 2019).

CONCLUSION

The results of this study were in accordance with the literature. According to the results, it was determined that in ART procedures individuals with CT genotype for the *VEGFR2* gene rs2305948 region, develop much higher number of follicles and these individuals are also at risk for developing OHSS. This variant rs2305948 may be a genetic marker for detecting women who are poor and high responders before ovulation stimulation. Further and larger studies are needed in order to confirm these results.

RECOMMENDATIONS

There is a need for more comprehensive prospective and randomised studies in order to clarify the association of *VEGFR2* gene mutations with OHSS or exaggerated ovarian response.

ACKNOWLEDGEMENT

The researchers would like to thank Ondokuz Mayis University BAP for supporting the project (PYO.TIP.1904.15.028).

ABBREVIATIONS

ART: Assisted reproductive technology
COH: Multiple follicle development with controlled ovarian hyperstimulation
IVF: In vitro fertilisation
ICSI: Intracytoplasmic sperm injection
PCR: Polymerase Chain Reaction
VEGF: Vascular Endothelial Growth Factor

REFERENCES

Abedal-Majed MA, Kurz SG, Springman SA, McNeel AK, Freetly HC, Largen V, Magamage M, Sargent KM, Wood JR, Cushman RA 2019. Vascular endothelial growth factor A isoforms modulate follicle development in peripubertal heifers independent of diet through diverse signal transduction pathways. *Biology of Reproducion*, ioz211. doi.org/10.1093/biolre/ioz211.

Altmäe S, Hovatta O, Stavreus-Evers A, Salumets A 2011. Genetic predictors of controlled ovarian hyperstimulation: Where do we stand today? *Hum Reprod Update*, 17: 813–828.

Atalay MA, Ugurlu N, Zulfikaroglu E, Danisman N 2016. Clinical significance of maternal serum vascular endothelial growth factor (VEGF) level in idiopathic recurrent pregnancy loss. *Eur Rev Med Pharmacol Sci*, 20: 2974-2982.

Bhanoori M, Arvind Babu K, Pavankumar Reddy NG, Lakshmi Rao K, Zondervan K, Deenadayal M et al. 2005. The vascular endothelial growth factor (VEGF) +405G>C 52 -untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study. *Hum Reprod*, 20: 1844–1849.

Dan W, Jing G, Liangbin X, Ting Z, Ying Z 2015. Association of follicle stimulating hormone receptor promoter with ovarian response in IVF-ET patients. *Iran J Reprod Med*, 11: 715-720.

Dean AG, Sullivan KM, Soe MM 2010. Open Source Epidemiologic Statistics for Public Health, Version 2.3.1www. From<OpenEpi.com> (Retrieved on 21 February 2011).

Enskog A, Nilsson L, Brännström M 2001. Plasma levels of free vascular endothelial growth factor (165) (VEGF(165)) are not elevated during gonadotropin stimulation in in vitro fertilization (IVF) patients developing ovarian hyperstimulation syndrome (OHSS): Results of a prospective cohort study with matched controls. *Eur J Obstet Gynecol Reprod Biol*, 96: 196–201.

Fabregues F, Balasch J, Manau D, Jimenez W, Arroyo V, Creus M et al. 1998. Hematocrit, leukocyte and platelet counts and the severity of the ovarian hyperstimulation syndrome. *Hum Reprod*, 13: 2406-2410.

Fang AM, Lee AY, Kulkarni M, Osborn MP, Brantley MA 2009. Polymorphisms in the VEGFA and VEGFR-2 genes and neovascular age-related macular degeneration. *Mol Vis*, 15: 2710–2719.

Ferrara N 2004. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev*, 25: 581–611.

Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA 2010. Single nucleotide polymorphisms in the anti-Müllerian hormone signalling pathway do not determine high or low response to ovarian stimulation. *Reprod Biomed Online*, 21(5): 616–623.

Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA 2012. Increased risk of ovarian hyperstimulation syndrome following controlled ovarian hyperstimulation in patients with vascular endothelial growth factor +405 cc genotype. *Gynecol Endocrinol*, 28: 845–849.

Kaczmarek MM, Kowalczyk AE, Waclawik A, Schams D, Ziecik AJ. 2007. Expression of vascular endothelial growth factor and its receptors in the porcine corpus luteum during the estrous cycle and early pregnancy. *Mol Reprod Dev*, 74: 730–739.

Kariyazono H, Ohno T, Khajoee V, Ihara K, Kusuhara K, Kinukawa N et al. 2004. Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphisms with coronary artery lesions of Kawasaki disease. *Pediatr Res*, 56: 953–959.

Laleethambika N, Mahfrid N, Dharwadkar, Santhy KS, Sangeetha M, Silambuchelvi D, Balachandar V 2016. Evaluation of M2/ANXA5 haplotype and P53 Codon 72 polymorphism in a patient with recurrent pregnancy loss, ectopic pregnancy and recurrent implantation failure. *International Journal of Human Genetics*,16(3, 4): 164-169.

Lledo B, Turienzo A, Ortiz JA, Morales R, Ten J, Llácer J, Bernabeu R 2014. Negative effect of P72 polymorphism on p53 gene in IVF outcome in patients with repeated implantation failure and pregnancy loss. *J Assist Reprod Genet*, 31: 169–172.

McClure N, Healy DL, Rogers PA, Sullivan J, Beaton L, Haning RV Jr et al. 1994. Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. *Lancet*, 344: 235–236.

Nouri K, Haslinger P, Szabo L, Sator M, Schreiber M, Schneeberger C et al. 2014. Polymorphisms of VEGF and VEGF receptors are associated with the occurrence of ovarian hyperstimulation syndrome (OHSS)—a retrospective case–control study. *Journal of Ovarian Research*, 7: 54.

O'Brien TJ, Harralson AF, Tran T, Gindoff I, Orkunoglu-Suer FE, Frankfurter D et al. 2014. Kinase insert domain receptor/vascular endothelial growth factor receptor 2 (KDR) genetic variation is associated with ovarian hyperstimulation syndrome. *Reprod Biol Endocrinol*, 12: 36.

Offical Homepage of NCBI 2020. From <https://www.ncbi.nlm.nih.gov/gene/3791> (Retrieved on 22 March 2020).

Pau E, Alonso-Muriel I, Gómez R, Novella E, Ruiz A, García- Velasco JA, Simón C, Pellicer A 2006. Plasma levels of soluble vascular endothelial growth factor receptor-1 may determine the onset of early

and late ovarian hyperstimulation syndrome. *Hum Reprod*, 21: 1453–1460.

Roque M, Bianco B, Christofolini D, Cordts E, Vilarino F et al. 2018. Pharmacogenetic algorithm for individualized controlled ovarian stimulation (iCOS) in assisted reproductive technology cycles. *Panminerva Med*. doi: 10.23736/S0031-0808.18.03496-1

Steffensen KD, Waldstrøm M, Brandslund I, Jakobsen A 2010. The relationship of VEGF polymorphisms with serum VEGF levels and progression-free survival in patients with epithelial ovarian cancer. *Gynecol Oncol*, 117: 109-116.

Taha S, Gamal SM, Nabil M, Naeem N, Labib D, Siam I et al. 2016. Vascular endothelial growth factor G1612A (rs10434) gene polymorphism and neuropsychiatric manifestations in systemic lupus erythematosus patients. *Rev Bras Reumatol*, 57(2): 149-153. doi: 10.1016/j.rbr.2016.08.005.

Tural S, Tekcan A, Kara N, Elbistan M, Güven D, Ali Tasdemir H 2015. FMR1 gene mutation screening by TP-PCR in patients with premature ovarian failure and fragile-X. *Gynecol Endocrinol*, 31(3): 191-195. doi: 10.3109/09513590.2014.975685.

Wang Y, Zheng Y, Zhang W, Yu H, Lou K, Zhang Y et al. 2007. Polymorphisms of KDR gene are associated with coronary heart disease. *J Am Coll Cardiol*, 50: 760-767.

Yueniwati Y, Darmiastini NK, Arisetijono E 2016. Thicker carotid intima-media thickness and increased plasma VEGF levels suffered by post-acute thrombotic stroke patients. *Int J Gen Med*, 14(9): 447-452. doi: 10.2147/IJGM.S114577.

Zhang W, Sun K, Zhen Y, Wang D, Wang Y, Chen J et al. 2009. VEGF receptor-2 variants are associated with susceptibility to stroke and recurrence. *Stroke*, 40: 2720–2726.

Zou Fuqin, Zeng Dong, Wen Bin et al. 2016. Illumina Miseq platform analysis caecum bacterial communities of rex rabbits fed with different antibiotics *AMB Express*, 6: 100.

Paper received for publication in December, 2019
Paper accepted for publication in March, 2020