Original Article

Down-Regulation of miR-93 Negatively Correlates with Overexpression of *VEGFA* and *MMP3* in Endometriosis: A Cross-Sectional Study

Raden Muharam, Ph.D.[1, 2, 3*], Ririn Rahmala Febri, M.Sc.[2], Kresna Mutia, M.Sc.[2], Pritta Ameilia Iffanolida, M.Sc.[2], Mila Maidarti, Ph.D.[1, 2, 3], Budi Wiweko, Ph.D.[1, 2, 3], Andon Hestiantoro, Ph.D.[1, 2, 3]

1. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
2. Human Reproduction, Infertility, and Family Planning Research Center, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
3. Yasmin IVF Clinic, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

Abstract

Background: Endometriosis is identified as presence of the endometrium outside the uterine cavity. Retrograde menstruation contributes to the endometrial tissue implantation and the establishment of endometriotic lesions at ectopic sites. It has been suggested that the endometriotic lesions are rich in angiogenic growth factors, while they have an essential role in survival and invasion of these cells. We investigated regulation of microRNA-93 (miR-93) and its involvement with vascular endothelial growth factor A (*VEGFA*) and matrix metalloproteinase (*MMP*) 3 expression in women with endometriosis.

Materials and Methods: This was a cross-sectional study at Central Surgical Installation, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, between October 2020 and November 2021. Eutopic and ectopic endometrial tissues were collected from 30 subjects with laparoscopically-confirmed endometriotic women. Normal endometrial cells of non-endometriosis women served as controls. Total RNA was isolated from all samples and a quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to analyze the expression of *miR-93*, *VEGFA* and *MMP3*.

Results: There was no significant difference in the expression levels of *VEGFA* (2.14 ± 0.50, P=0.719) and *MMP3* (2.99 ± 0.42, P=0.583) between endometriotic lesions of endometriosis women and the healthy endometrium. Expression of miR-93 was significantly lower in the eutopic endometrium (16.7 fold) and ectopic endometriotic lesion (20 fold) compared to the normal endometrium (P<0.001). Furthermore, we also observed a significant correlation between *miR-93* and *VEGFA* expression in eutopic endometrium obtained from women with endometriosis (r=-0.544, P=0.029). Expression of the *miR-93* was also negatively correlated with MMP3 expression in both eutopic (r=-0.412, P=0.01) and ectopic (r=-0.539, P=0.03) endometrial cells of women with endometriosis.

Conclusion: *VEGFA* and *MMP3* expression levels trended to be increased in both eutopic and ectopic endometrial tissues of endometriosis women, while down-regulation of miR-93 might be involved in the alteration of *VEGFA* and *MMP3* in endometriosis.

Keywords: Angiogenesis, Endometriosis, miR-93, *MMP3*, *VEGFA*

Citation: Muharam R, Rahmala Febri R, Mutia K, Ameilia Iffanolida P, Maidarti M, Wiweko B, Hestiantoro A. Down-regulation of miR-93 negatively correlates with overexpression of VEGFA and MMP3 in endometriosis: a cross-sectional study. Int J Fertil Steril. 2023; 17(1): 28-33. doi: 10.22074/IJFS.2022.543884.1233.
This open-access article has been published under the terms of the Creative Commons Attribution Non-Commercial 3.0 (CC BY-NC 3.0).

Introduction

Endometriosis is a chronic and inflammatory reproductive disorder, specified by presence of the endometrial cells like lesions outside of the uterus (1). The current understanding of etiology of endometriosis is yet uncertain. Retrograde menstruation theory proposed by Sampson in 1927 is suggested to contribute to the endometrial tissue implantation and endometriotic lesions development at ectopic sites (2). However, although approximately 90% of menstruating women experience retrograde menstruation, only 5-10% are at risk of developing endometrial lesions (3). Thus, several factors might be involved in the implantation and growth of this ectopic endometrium as endometrial cells implantation requires neovascularization to establish, grow and invade tissues. From the pathophysiological point of view, it is indicated that the factors playing an essential role in the survival and invasion of ectopic endometrial cells are rich

Received: 29/November/2021, Revised: 04/April/2022, Accepted: 11/April/2022
*Corresponding Address: Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
Email: rmuharam@yahoo.com

angiogenic factors in these cells, while they work together with metalloproteinases (MMPs) (4).

Angiogenesis is an essential step in endometriotic lesion formations, since the ectopic survival of the endometrium requires formation of new blood vessels. Vascular endothelial growth factor (VEGF), as a heparin-binding glycoprotein, is an important angiogenic factor that stimulates proliferation and migration of endothelial cells and increases vascular permeability (5). Several studies reported that VEGF concentration was significantly increased in the peritoneal fluid of endometriosis women compared to non-endometriosis (6, 7). These results suggested that VEGF is also involved in the angiogenesis of endometriosis. In addition, ectopic endometrial fragments also require alteration in the expression of molecules responsible for tissue invasion, such as over-expressed MMPs (8). These proteolytic enzymes are involved in normal endometrial remodeling, which occurs during the cellular proliferative phase at the time of menstrual bleeding. MMP3 also contributes to the invasion of endometrial cells toward the outside of uterine environment, as well as tissue remodeling, forming an endometriotic lesion (9).

Recent studies indicated that dysregulation of microRNAs (miRNAs) emerged essential in endometriosis development (10). miRNAs are small, noncoding, highly conserved RNA molecules consisting of 19-24 nucleotides. They contribute to controlling translation and stability of targeted RNA bases to complement sites and promote repression or degradation of messenger RNA transcripts (11). Burney et al. (12) evaluated miR-34c-5p, miR-34c-3p, miR-9 and miR-34b expression levels and found that expression of these miRNAs were downregulated in ectopic endometrium from women with endometriosis, compared to women without endometriosis. These miRNAs affected stability of the expression of their target genes and played an essential role in the pathogenesis of endometriosis. Furthermore, several miRNAs were also categorized as proangiogenic miRNAs, playing role in the regulation of vascular endothelial growth factor A (VEGFA) (13, 14) and MMP3 translations (15). In general, VEGFA mRNA is targeted by miR-93, resulting in cells proliferation inhibition (16). Additionally, increase of endothelial cell migration and tube formation is also mediated by miR-93. However, in endometriosis, it is found that low level of miR-93 is associated with high level of VEGFA in endometriosis. Here, we investigated whether alteration of miR-93 was involved in overexpression of VEGFA and MMP3 in both eutopic endometrial and endometriotic tissues of women with endometriosis.

Materials and Methods

Sample collection

This was a cross-sectional study at Central Surgical Installasion (Dr. Cipto Mangunkusumo General Hospital; Jakarta, Indonesia) between October 2020 and November 2021. Thirty women (mean age 33.89 ± 5.33 years) diagnosed with endometriosis using ultrasonography were recruited as cases to this study. The endometriosis stages were classified based on the revised American Society for Reproductive Medicine (ASRM) scoring system: stages I, II, III, and IV (17). We surgically collected 30 eutopic endometrial and 30 endometriotic tissues, while tissues of the both groups were obtained from women with endometriosis. Healthy controls (n=30, mean age 36.27 ± 6.79 years) were enrolled from women who underwent in vitro fertilization procedure in the same hospital and had no gynecologic disorders. None of these groups received hormonal drugs in the last three months. Endometriosis tissues were collected using laparoscopy method. Meanwhile, normal endometrial tissues were collected using a sterile pipelle cannula CCD (Pipelle de Cornier, Laboratoire CCD; Paris, France).

All participants signed the informed consent prior to the tissue collection. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, University of Indonesia - Cipto Mangunkusumo Hospital (KET-972/UN2.F1/ETIK/PPM.00.02/2020).

Total RNA extraction and Complementary DNA synthesis

Total RNA was extracted from all tissues using Tissue Total RNA Mini Kit (Geneaid, Taiwan) according to the manufacturer's protocol. Quality and quantity of RNA was measured by NanoPhotometer Implen P300 (Implen GmbH, Germany). ReverTra Ace™ qPCR RT Master Mix (Toyobo Co., Japan) was used for complementary DNA (cDNA) synthesis. A suitable amount of RNA template (6 µl, 50 ng/µl) was prepared and followed by denaturation at 65°C for 5 minutes. RNA template was mixed with 2 µl of 4x DN Master Mix and incubated at 37°C for 5 minutes. Then, 5x RT Master Mix II was added to the mixture and placed in the thermal cycler with the following condition: 37°C for 15 minutes, 50°C for 5 minutes and 98°C for 5 minutes.

Quantitative reverse-transcription polymerase chain reaction for mRNA expression

Pairs of primer targeting the selected (*VEGFA* and *MMP3*) and reference (actin beta; *ACTB*) genes were designed using the software platform Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Table 1). SensiFAST™ SYBR® No-ROX Kit (Meridian Bioscience, USA) was used for performing quantitative reveres transcription polymerase chain reaction (qRT-PCR). Each reaction contains 10 µl of 2x master mix, 0.5 µl (20 µM) of each primer, 3 µl of cDNA template and 6 µl nuclease free water. An initial denaturation step was run at 95°C for 2 minutes, continued by 40 cycles of amplification with denaturation at 95°C for 5 seconds, annealing at 55°C for 10 seconds and extension at 72°C for 15 seconds. qRT-PCR was carried out using Prime Pro 48 Real-Time PCR (Techne Cole-Parmer, UK). Melt curve analysis was used to assess whether the qRT-PCR assays have generated a single specific product. The experiment was carried out twice for each sample.

Table 1: Primers sequence used for mRNA expression

Gene	Primer sequences (5´-3´)	Size (bp)	Tm (°C)
VEGFA	F: GAGGAGTCCAACATCACCAT R: GCATTCACATTTGTTGTGCT	99	55
MMP3	F: GGTCACTTCAGAACCTTTCC R: CTTTTGGCAAATCTGGTGTA	91	55
ACTB	F: CACTCTTCCAGCCTTCCTTC R: GTACAGGTCTTTGCGGATGT	104	55

Quantification of miRNAs

Specific miRNA sequences for hsa-mir-93 and U6 small nuclear (snRNA) were obtained from the microRNA database (https://www.mirbase.org). Mature miRNA was reverse transcribed to single-stranded cDNA using the Taqman® MicroRNA Reverse Transcription Kit and Taqman™ MicroRNA Assays has-mir-93 (Applied Biosystems, USA). The cDNA was amplified using Taqman® Fast Advanced Master Mix (Applied Biosystems). Reaction mixture included cDNA template (1:10 dilution), miRNA assay (20x), master mix (2x) and nuclease-free water, for final volume of 10 µl. The cycling condition was initiated by polymerase activation at 95°C for 20 seconds and then 40 cycles at 95°C for 3 seconds and annealing at 60°C for 30 seconds. Expression of target miR-93 was normalized using expression of the reference miRNA, U6 snRNA. The experiment was carried out twice for each sample.

Statistical analysis

Data were collected with Pro Study Software (Techne Cole-Parmer, UK) using linear baseline correlation method and global auto cycle threshold (Ct) method. Then, Ct values were normalized using mean expression of the housekeeping genes selected. Relative mRNA and miRNA expression were presented using the formula $2^{-\Delta\Delta Ct}$ (Livak method) (18). Fold change was calculated to determine upregulation and downregulation of the target genes. The Prism 5 software (GraphPad software, USA) was used for all statistical analysis. One-way ANOVA test was used to determine difference of *VEGFA* and *MMP3* mRNA with miRNA-93 expressions between ectopic and eutopic tissues from women suffering endometriosis and normal endometrial tissues. The correlation of miRNA-93 with mRNA (VEGFA and MMP3) expressions in the endometriotic tissues were assessed using Pearson's correlation. The level of significant was set as 5%.

Results

Upregulation of VEGFA and MMP3 expression in endometriosis

We performed qRT-PCR to determine alteration of *VEGFA* and *MMP3* expression in the eutopic endometrium and ectopic endometriosis tissues collected from women suffering endometriosis in comparison with normal endometrium from healthy controls. As shown in Figure 1, no significant difference was seen in the expression levels of *VEGFA* and *MMP3* between eutopic and ectopic endometriosis tissues of women with endometriosis compared to the heathy endometrium. However, expression of VEGFA was increased by 0.66 fold in the eutopic endometrium and 1.14 fold in the endometriotic lesion from endometriosis women compared to the normal endometrium. In addition, an increased expression level of *MMP3* was observed in the endometriotic lesion from endometriosis women compared to the normal endometrium, by 2 fold and 1.99 fold, respectively.

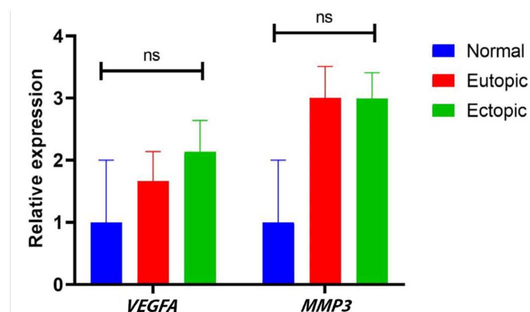

Fig.1: Relative mRNA expression levels of *VEGFA* and *MMP3* in the ectopic endometriosis lesions, eutopic endometrium and normal endometrium. The y-axis represents fold change in expression level as determine by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), while it is expressed as mean ± SE. The difference of *VEGFA* and *MMP3* mRNA expressions in three groups was analyzed using One-way ANOVA test. ns; Not significant.

Expression of *miR-93* was decreased in women with endometriosis

Similar to *VEGFA* and *MMP3* expressions, we examined expression level of *miR-93* in three sample groups using qRT-PCR. We observed that expression of *miRNA-93* was significantly decreased in the eutopic endometrium (16.7 fold) and ectopic endometriotic lesion (20 fold) compared to the normal endometrium (P<0.001, Fig.2).

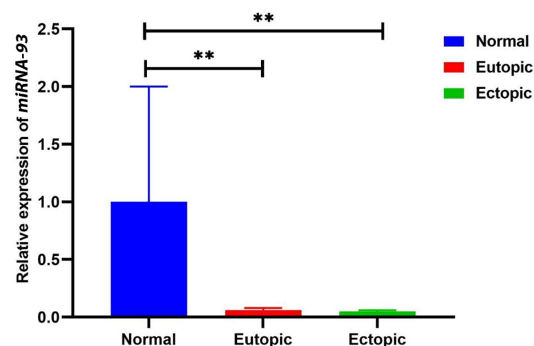

Fig.2: Relative expression level of *miRNA-93* in the ectopic lesions, eutopic endometrium and normal endometrium. The y-axis represents fold change in expression level, as determine by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), while it is expressed as mean ± SE. The difference of *miRNA-93* expression levels in three groups was analyzed using One-way ANOVA test. **; P<0.01 was considered statistically significant.

mRNA expression levels of *VEGFA* and *MMP3* based on menstrual phases

We also analyzed *VEGFA* and *MMP3* mRNA expression levels in the eutopic endometrium and endometriotic lesions obtained from women suffering endometriosis based on the menstrual phase: the

proliferative and secretory phases. The results showed that there was no difference in the mRNA expression levels of *VEGFA* and *MMP3* in the eutopic endometrium and endometriotic lesions of both menstrual cycle phases (Table 2)

Table 2: *VEGFA* and *MMP3* expressions in eutopic and ectopic of women with endometriosis in menstrual cycle

Variables	Proliferative (n=16)	Secretory (n=14)	P value
Eutopic endometrium			
VEGFA	1.35 ± 0.51	2.18 ± 0.97	0.887
MMP3	3.15 ± 0.70	2.73 ± 0.78	0.707
Ectopic endometrium			
VEGFA	2.13 ± 1.13	2.13 ± 0.50	0.749
MMP3	3.22 ± 0.54	2.62 ± 0.70	0.508

The values are expressed as mean ± SE. T-independent test.

mRNA expression levels of *VEGFA* and *MMP3* based on the stage of endometriosis

In this study, endometriosis was laparoscopically confirmed in 30 women of revised ASRM stage II (n=3), stage III (n=9) and stage IV (n=18). However, we found no difference in the *VEGFA* and *MMP3* mRNA expression levels of both eutopic and ectopic tissues based on the stages of endometriosis (Table 3).

Table 3: *VEGFA* and *MMP3* expression levels in eutopic endometrium and ectopic tissues based on endometriosis stages

Variables	Stage II (n=3)	Stage III (n=9)	Stage IV (n=18)	P value
Eutopic endometrium				
VEGFA	1.90 ± 1.56	1.74 ± 1.02	1.55 ± 0.62	0.631
MMP3	2.14 ± 0.85	3.88 ± 1.20	2.89 ± 0.71	0.559
Ectopic endometrium				
VEGFA	2.09 ± 0.45	1.88 ± 0.82	2.26 ± 0.84	0.769
MMP3	3.30 ± 0.34	3.15 ± 0.81	2.82 ± 0.68	0.905

The values are expressed as mean ± SE. One-way ANOVA test. P<0.05 was considered to indicate statistically significance.

Expression of *miR-93* based on the endometriosis stage

We also analyzed expression of *miR-93* in different stages of endometriosis. According to the statistical analysis, we found no difference in the expression levels of *miR-93* based on the stages of endometriosis in both eutopic and ectopic tissues (Table 4).

Table 4: *miR-93* expression in eutopic endometrium and ectopic tissue based on endometriosis stages

Variables	Stage II (n=3)	Stage III (n=9)	Stage IV (n=18)	P value
Eutopic endometrium	0.04 ± 0.04	0.07 ± 0.04	0.05 ± 0.03	0.545
Ectopic endometrium	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.913

The value are expressed in mean ± SE. One-way ANOVA test. P<0.05 was considered to indicate statistically significance.

The correlation between miRNA and mRNA

Correlation analysis was also performed to determine whether *VEGFA* and *MMP3* mRNA expression levels was upregulated by *miR-93*. We found that there was significantly negative correlation between the expression levels of *miR-93* and *MMP3* in endometrial cells and endometriotic lesions collected from endometriosis women (r=-0.544, P<0.05, r=-0.749, P<0.01, respectively). In addition, significantly negative correlation was observed in ectopic endometrium (r=-0.539, P<0.05), but not eutopic lesions of women with endometriosis.

Discussion

In the present study, we compared mRNA expression levels of *VEGFA* and *MMP3* in endometrial cells and endometriotic lesions between women with endometriosis and those without endometriosis using qRT-PCR. The result showed that mRNA of *VEGFA* was enhanced in both endometrial cells and endometriotic lesions from endometriosis women than normal endometrial tissue of non-endometriosis women although we observed no significant difference. In agreement with our study, Yerlikaya et al. (19) reported no significant difference in *VEGFA* expression levels of eutopic and ectopic endometriums. However, it was seen an increased trend of *VEGFA* expression in ectopic lesions. In contrast to our study, a study by performed by Takehara et al. (20) showed that *VEGFA* gene expression was significantly higher in endometriotic lesions obtained from peritoneal of women with endometriosis, than those without endometriosis.

The invasiveness of endometrial cells is similar to cancer cells (21). One of these invasive properties is related to the increased proteolytic activity of the tissue, resulting in the development of advanced endometriosis. In this case, overexpression of MMPs is believed to be related to the aggressive behavior of the endometrial cells (22) . However, we found no significant difference in the mRNA expression level of *MMP3* between women with endometriosis and those without endometriosis. The increasing *MMP3* mRNA expression level was also observed in this study, where *MMP3* mRNA expression level was two times higher in endometriotic lesions and endometrium of endometriosis patients than normal endometrium. Luddi et al. (9) studied both pro- and active *MMP3*. It was determined that their expression levels were significantly increased in endometriotic lesions compared to the normal endometrial cells. Consistent with these findings, Lv et al. (23) showed MMP3 expression levels were significantly higher in endometriosis compared to non-endometriosis. These results indicated that overexpression of *MMP3* is involved in the invasion of endometrial tissue to develop endometriotic lesions outside the uterine environment.

According to the implantation theory, eutopic endometrium can survive and develop into endometrial lesions in the peritoneal cavity due to retrograde menstruation (24). Hence, this study also examined

differences in *VEGFA* and *MMP3* expression level of the both eutopic and ectopic endometrial tissues of women with endometriosis, based on the menstrual phase and stage of endometriosis. Our results showed no difference in the *VEGFA* and *MMP3* expression levels between eutopic and ectopic endometrial based on menstrual cycle and endometriosis stage. In line with our results, Yerlikaya et al. (19) revealed no difference in the expression of *VEGFA* with the menstrual cycle. In addition, Augoulea et al. (25) showed that expression level of *VEGFA* was not changed in all stages of endometriosis.

Several studies have focused on the expression profiling of miRNAs in endometriosis, further to their coordination in angiogenesis and invasion of endometrial cells to the peritoneal environment (10). In the present study, we also measured miR-93 expression and revealed that miR-93 expression level in both eutopic and ectopic endometrial tissues from endometriosis women was significantly decreased in comparison with healthy controls. Some studies also reported that the expression of *miR-93* was downregulated in human cancer cells. Beside, high expression of *miR-93* suppressed proliferation and inhibited tumorigenesis in cancer (26, 27). Furthermore, we analyzed correlation of *miR-93* with *VEGFA* and *MMP3*. A negative correlation was observed between miR-93 expression level and *VEGFA* in endometriotic lesions. In addition, *miR-93* expression was inversely correlated with *MMP3* expression in both eutopic and ectopic endometrial cells of women suffering endometriosis. Previous studies performed by Lv et al. (23) was in line with our findings that indicated the downregulation of *miR-93* was negatively correlated with the increased expression levels of *VEGFA* and *MMP3* in endometriosis.

Conclusion

Increased expression levels of *VEGFA* and *MMP3* were observed in endometriosis compared to non-endometriosis. Additionally, decreased expression level of *miR-93* was negatively correlated with high expression of *VEGFA* and *MMP3* in both eutopic and ectopic endometrial cells of women with endometriosis. These findings could affect expression of angiogenic factors and play a role in the pathogenesis of endometriosis. However, further investigations are required to examine the role of *miR-93* in the post-transcriptional regulation of target mRNA in endometriosis.

Acknowledgements

The authors would like to thank Universitas Indonesia for funding this research through PUTI grant with contract NKB-1538/UN2.RST/HKP.05.00/2020. The authors declare no conflict of interest.

Authors' Contributions

R.M., A.H.; Participated in conception, study design, manuscript preparation, finalization of the manuscript, and approved the final draft. R.R.F., K.M., P.A.I.; RNA isolation, cDNA synthesis, real-time PCR analysis, and data analysis, manuscript preparation. M.M., B.W.; Data evaluation, provided critical revision, and approved the final draft. All authors read and approved the final manuscript.

References

1. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet. 2010; 27(8): 441-447.
2. Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol. 1927; 3(2): 93-110.
3. Vinatier D, Orazi G, Cosson M, Dufour P. Theories of endometriosis. Eur J Obstet Gynecol Reprod Biol. 2001; 96(1): 21-34.
4. Nap AW, Groothuis PG, Demir AY, Evers JL, Dunselman GA. Pathogenesis of endometriosis. Best Pract Res Clin Obstet Gynaecol. 2004; 18(2): 233-244.
5. Gargett CE, Lederman F, Heryanto B, Gambino LS, Rogers PA. Focal vascular endothelial growth factor correlates with angiogenesis in human endometrium. Role of intravascular neutrophils. Hum Reprod. 2001; 16(6): 1065-1075.
6. Wang H, Gorpudolo N, Li Y, Feng D, Wang Z, Zhang Y. Elevated vascular endothelia growth factor-A in the serum and peritoneal fluid of patients with endometriosis. J Huazhong Univ Sci Technolog Med Sci. 2009; 29(5): 637-641.
7. Kianpour M, Nematbakhsh M, Ahmadi SM, Jafarzadeh M, Hajjarian M, Pezeshki Z, et al. Serum and peritoneal fluid levels of vascular endothelial growth factor in women with endometriosis. Int J Fertil Steril. 2013; 7(2): 96-99.
8. Christodoulakos G, Augoulea A, Lambrinoudaki I, Sioulas V, Creatsas G. Pathogenesis of endometriosis : the role of defective ' immunosurveillance .' Eur J Contracept Reprod Heal Care. 2007; 12: 194-202.
9. Luddi A, Marrocco C, Governini L, Semplici B, Pavone V, Luisi S, et al. Expression of matrix metalloproteinases and their Inhibitors in endometrium : high levels in endometriotic lesions. Int J Mol Sci. 2020; 21(8): 2840.
10. Braza-boils A, Alexandre-Marı J, Gilabert J, Sanchez-Izquierdo D, Espana F, Estelles A, et al. MicroRNA expression profile in endometriosis : its relation to angiogenesis and fibrinolytic factors. Hum Reprod. 2014; 29(5): 978-988.
11. Guo SW. Epigenetics of endometriosis. Mol Hum Reprod. 2009; 15(10): 587-607.
12. Burney RO, Hamilton AE, Aghajanova L, Vo KC, Nezhat CN, Lessey BA, et al. MicroRNA expression profiling of eutopic secretory endometrium in women with versus without endometriosis. Mol Hum Reprod. 2009; 15(10): 625-631.
13. Liu B, Ding J, Luo J, Lu L, Yang F, Tan X. Seven protective miRNA signatures for prognosis of cervical cancer. Oncotarget. 2016; 7(35): 56690-56698.
14. Liu H, Xing A, Chen X, Ma R, Wang Y, Shi D. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. Oncotarget. 2015; 6(35): 37458-37470.
15. Chu C, Liu X, Bai X, Zhao T, Wang M, Xu R, et al. MiR-519d suppresses breast cancer tumorigenesis and metastasis via targeting MMP3. Int J Biol Sci. 2018; 14(2): 228-236.
16. Li F, Liang X, Chen Y, Li S. Role of microRNA-93 in regulation of angiogenesis. Tumor Biol. 2014; 35(11): 10609-10613.
17. Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, et al. World endometriosis society consensus on the classification of endometriosis. Hum Reprod. 2017; 32(2): 315-324.
18. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res. 1994; 6(10): 986-994.
19. Yerlikaya G, Balendran S, Pröstling K, Reischer T, Birner P, Wenzl R, et al. Comprehensive study of angiogenic factors in women with endometriosis compared to women without endometriosis. Eur J Obstet Gynecol Reprod Biol. 2016; 204: 88-98.
20. Takehara M, Ueda M, Yamashita Y. Vascular endothelial growth factor A and C gene expression in endometriosis. Hum Pathol. 2004; 35(1): 1369-1375.
21. Banu S, Lee J, Speights V, Starzinki-Powits A, Arosh J. Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms.

Endocrinology. 2008; 149(3): 1180-1189.
22. Barbe AM, Berbets AM, Davydenko IS, Koval HD, Barbe AM. Expression and significance of matrix metalloproteinase-2 and matrix metalloproteinas-9 in endometriosis material and methods subjects. J Med Life. 2020; 13(3): 314-320.
23. Lv X, Chen P, Liu W. Down regulation of MiR-93 contributes to endometriosis through targeting MMP3 and VEGFA. Am J Cancer Res. 2015; 5(5): 1706-1717.
24. Yovich JL, Rowlands PK, Lingham S, Sillender M, Srinivasan S. Pathogenesis of endometriosis: look no further than John Sampson. Reprod Biomed Online. 2020; 40(1): 7-11.
25. Augoulea A, Kindis A, Karopoulou E, Tsoltos N, Kaparos G, Tsakonas E, et al. Age at menarche and oxidative stress markers in women with endometriosis. SN Compr Clin Med. 2020; 2: 69-74.
26. Yu X, Zou J, Bao Z, Dong J. miR-93 suppresses proliferation and colony formation of human colon cancer stem cells. World J Gastroenterol. 2011; 17(42): 4711-4717.
27. Yang I, Tsai H, Hou M, Chen K, Tsai P, Huang S, et al. MicroRNA-93 inhibits tumor growth and early relapse of human colorectal cancer by affecting genes involved in the cell cycle. Carcinogenes. 2012; 33(8): 1522-1530.