



# Polymorphisms of Estrogen Receptor- $\alpha$ and Estrogen Receptor- $\beta$ Genes and its Expression in Endometriosis

## Östrojen Reseptör- $\alpha$ ve Östrojen Reseptör- $\beta$ Genlerinin Polimorfizmi ve Endometriozisde İfadelenmeleri

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### ABSTRACT

**Objectives:** Endometriosis is a common gynecological disorder, characterized by the presence of endometrial-like tissue in the extrauterine location. The increasing estradiol concentration can influence endometriosis risk and estrogen receptor (ER) activity. Polymorphism in ER causes gene expression alteration and influences hormone-receptor interaction. This research aims to determine ER genetic polymorphisms in endometriosis pathogenesis.

**Materials and Methods:** This study was performed on case-control polymorphisms, which compared 83 women with endometriosis and 76 women without endometriosis. However, the samples used for *ER* gene expression analysis and estrogen level measurement were obtained from 18 women with endometriosis and 18 women without endometriosis. Polymerase chain reaction-restriction fragment length polymorphism was used to determine ER genetic polymorphisms. Chi-square, Mann-Whitney test, Spearman's correlation ( $\rho$ ), t-independent, and two-tailed tests were used to analyze the data.

**Results:** Association between the allele *ER $\alpha$*  rs9340799 A/G and endometriosis was significantly different ( $p=0.012$ ), whereas rs2234693 T/C polymorphism showed no association with endometriosis. The correlation between the genotype frequencies of allele *ER $\beta$*  rs4986938 G/A and endometriosis was found significantly different ( $p=0.015$ ;  $p=0.034$ ).

**Conclusion:** Estradiol level and *ER $\beta$*  expression increases, polymorphism genotypes and alleles of *ER $\beta$*  rs4986938 G/A gene and allele frequency of *ER $\alpha$*  rs9340799 A/G gene have roles in endometriosis.

**Key words:** Estradiol (E2), estrogen receptor (*ER $\alpha$*  and *ER $\beta$* ), endometriosis

### ÖZ

**Amaç:** Endometriozis, ekstrauterin lokasyonda endometrial benzeri dokunun varlığı ile karakterize yaygın bir jinekolojik hastalıktır. Östradiol konsantrasyonunun artması, endometriozis riskini ve östrojen reseptörlerinin (ER) aktivitesini etkileyebilir. ER'deki polimorfizm, gen ekspresyonunda değişikliğe neden olur ve hormon-reseptör etkileşimini etkiler. Bu araştırma, endometriozis patogeneğinde ER'nin genetik polimorfizmini belirlemeyi amaçlamaktadır.

**Gereç ve Yöntemler:** Bu olgu-kontrol çalışması, endometriozisi olan 83 kadın ile endometriozisi olmayan 76 kadını karşılaştıran bir polimorfizm çalışmasıdır. Ancak *ER* gen ekspresyon analizi ve östrojen düzeyi ölçümü için kullanılan örnekler 18 endometriozisi olan ve 18 endometriozisi olmayan kadından elde edildi. ER genetik polimorfizmlerini belirlemek için polimeraz zincir reaksiyonu restriksiyon parça uzunluğu polimorfizmi kullanıldı. Veriler ki-kare, Mann-Whitney test, Spearman rho, t-bağımsız ve iki-kuyruklu testleri kullanılarak analiz edildi.

**Bulgular:** Allel *ER $\alpha$*  rs9340799 A/G ile endometriozis arasındaki ilişki anlamlıydı ( $p=0,012$ ). Aksine, rs2234693 T/C polimorfizmi ile endometriozis arasında bir ilişki bulunamadı. Genotip ve allel *ER $\beta$*  rs4986938 G/A frekansları ile endometriozis arasındaki ilişki anlamlı bulundu ( $p=0,015$ ;  $p=0,034$ ).

**Sonuç:** Östradiol düzeyi ve *ER $\beta$*  ekspresyonu, polimorfizm genotipleri ve *ER $\beta$*  4986938 G/A geninin allelleri ve *ER $\alpha$*  rs9340799 A/G geninin allel sıklığı endometriozisde rol oynamaktadır.

**Anahtar kelimeler:** Estradiol (E2), östrojen reseptörü (*ER $\alpha$* , ve *ER $\beta$* ), endometriozis

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Received: 23.09.2019, Accepted: 26.12.2019

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## INTRODUCTION

Chronic gynecological disorder manifested by the presence of endometrium-like tissue that grows outside the uterine cavity is called endometriosis.<sup>1,2</sup> Endometriosis incidence is estimated at 10%-15%, and reached 13.6%-69.5% of the infertile group based on clinical data from various hospitals in Indonesia.<sup>2-4</sup> Endometriosis etiology and pathophysiology is not fully understood yet. The interaction of various factors, such as the environment, immunology, and genetics, causes a multifactorial disease called endometriosis.<sup>5,6</sup>

It is closely related to estrogen levels. Generally, patients with endometriosis have higher estrogen levels than normal women, which can stimulate endometrial tissue and stromal cell growth outside the uterine cavity, causing pain and inflammation. Estrogen levels in the serum are twice the highest peak of the proliferative phase (before ovulation) and the luteal phase based on the women menstrual cycle. Although high estradiol concentrations in the endometriosis tissues are known from previous studies, the level of serum estradiol ( $E_2$ ) peripheral in patients with the highest endometriosis, especially the proliferative phase, is still unknown.<sup>7</sup>

The biological activity of the estrogen is mediated by estrogen receptors (ERs), including ER $\alpha$  and ER $\beta$  therefore, changes in the quantity and the receptor affinity can cause a pathological condition. The two ER subtypes of ER $\alpha$  and ER $\beta$  are encoded by two different genes and chromosomes. ER $\alpha$  and ER $\beta$  are located in chromosome six locus q25.1 and chromosome 14 locus q22-24, respectively.<sup>8,9</sup> An increased risk of endometriosis is contributed by the ER $\alpha$  rs2234693 gene polymorphism, especially in the Caucasian group; and the ER $\alpha$  rs9340799 gene with GG genotype (mutant) contributes four times increasing endometriosis risk [OR: 4.67, confidence interval (CI): 95%: 1.84-11.83] with  $p=0.001$ , and 3 times causing the risk of IVF failure with  $p=0.018$  (OR: 3.33, CI 95%: 1.38-8.03).<sup>10</sup> Likewise in Brazil, ER $\beta$  gene polymorphism rs4986938 showed endometriosis risk and infertility risks.<sup>11</sup> ER gene polymorphism shows inconsistent results among countries/ethnicity. However, research on the ER gene characteristics, especially in patients with endometriosis in Indonesia, has not been studied yet.

This ER gene polymorphism was thought to contribute elevating or lowering the gene expression levels. A previous study reported that expression in ER $\beta$  endometriosis tissue increased 100 times compared with normal endometrial tissue. Moreover, ER $\beta$  suppressed ER $\alpha$  expression in endometriosis tissue; however, the mechanism is not clearly understood.<sup>12</sup> Therefore, further investigation on ER gene polymorphism in patients with endometriosis is required as well.

## MATERIALS AND METHODS

### Materials

The blood samples in this research were from 83 and 76 women with and without endometriosis, respectively. All samples were obtained from the Polyclinic Immuno-Endocrinology, Department of Obstetrics and Gynecology, RSCM-FKUI, RS Budhi Jaya, and RS Sammeri, and were approved by the

Ethical Committee of Universitas Indonesia (no: 165/PTO2.FK/ETIK/2010). ER $\alpha$  intron 1 gene polymorphisms rs9340799 and rs2234693 and rs4986938 of ER $\beta$  exon 8 were used.

### Isolation of DNA from peripheral blood

An amount of 4.5 mL of red blood cell 1x (199 mM EDTA, 100 mM KHCO<sub>3</sub>, 1.45 NH<sub>4</sub>Cl) was mixed into 1.5 mL of peripheral blood. It was then inverted and incubated for 10 min at room temperature. NF 400/NF400R Bench-Top Centrifuge was used to centrifuge the samples at 1500 rotations per minute (rpm) for 10 min at room temperature. The supernatant was slowly discarded, leaving the rest of the sediment in the next tube of leukocyte form. Cell lysis solution of 300 mL, which contained 10 mM Tris-HCl, 0.25 mM EDTA, and 20% sodium dodecyl sulfate, was placed in a tube, pipetted until it became homogeneous, then incubated in a water bath at 37°C for 30-60 min. A 300 mL precipitated protein solution containing 5 M ammonium acetate was added to the solution and homogenized using the Stuart Scientific Autovortex SA6 for 10-20 sec until brown granules were formed. Samples were centrifuged at 3000 rpm for 15 min at 4°C. The brown pellet form (protein) was discarded after centrifugation, and the supernatant containing DNA was collected. The supernatant was transferred in a new tube, which contained 2.3 mL of isopropanol cold solution, then inverted for approximately 25-30 times to get DNA materials. It was centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was discarded, and the pellet was washed with 1.3 mL of ethanol 70% sterile and centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was discarded, and the pellet (DNA) was dried in open air by turning the tube for approximately 2 h at room temperature or 1 h at 37°C. The DNA was rehydrated with 300 mL of TE solution (10 mM Tris-HCl and 0.25 EDTA) and incubated for 2 h at 37°C. It was then transferred to a 1.5 mL sterile tube. NanoDrop (Maestro) was used to measure the DNA's concentration and purity in the case and control groups. The solution is stored at -20°C until further examination.

### Amplification of DNA fragments of ER genes

Polymerase chain reaction (PCR) method and specific primers to target gene-based software primer 3 were used for DNA fragment amplification. The primer sequences used for ER $\alpha$  gene intron 1 amplification are 5'-CAG-GGT-TAT-GTG-GCA-AC-ATG-3' (forward) and 5'-TAC-CTA-TAA-TGA-CAA-AAA-AA-3' (reverse), whereas for genes ER $\beta$  exon 8 are 5'-CGG-CAG-AGG-ACA-GTA-AAA-GC-3' (forward) and 5'-AGG-CCA-TTG-AGT-GTG-GAA-AC-3' (reverse). Each reagent has a volume of 25  $\mu$ L, which consisted of 5  $\mu$ L genomic DNA, 12.5  $\mu$ L master mix (Kappa from Bionline), 0.5  $\mu$ L forward primer, 0.5  $\mu$ L reverse primer, and 6.5  $\mu$ L aquabidest (ddH<sub>2</sub>O).

At each gene, DNA samples were amplified for 35 cycles. PCR conditions used for ER $\alpha$  gene on the region intron 1 had an initial denaturation at 94°C for 6 min, went into a cycle consisting of denaturation at 94°C for 60 sec, annealing at 56°C for 40 sec, and elongation at 72°C for 90 sec. Elongation was at 72°C for 5 min at the end of the cycle. ER $\beta$  gene amplification stages in exon 8 and PCR ER $\alpha$  gene were almost the same; however,

the annealing temperature used was 54°C. Amplification results were separated by electrophoresis on 2% agarose gel containing ethidium bromide (0.5 mg/mL) in 1x TE buffer (0.04 M Tris-acetate and 0.002 M EDTA; pH 8.0) at 90V for 40 min. Amplicon sizes of *ERα* and *ERβ* genes were 255 bp and 293 bp, respectively.

*ER* gene polymorphism analysis with restriction fragment length polymorphism for *ERα* gene polymorphism in intron 1 rs9340799 known area by XbaI, rs2234693 with PvuII, and *ERβ* gene in exon 8 rs4986938 area with AluI. DNA cleavage was done by adding 0.5 μL (10U/μL) of enzymes XbaI, PvuII, or AluI (New England Biolabs) into tubes already containing 10.5 μL DNA fragment amplification product, 1 mL of buffer solution of ER 10x, and 9 mL ddH<sub>2</sub>O, with a total volume of 21 μL, and incubated in a water bath at 37°C for 4 h. DNA fragments were analyzed on a 3% agarose gel in 1x TE at 90V for 1 h. Fragment visualization with electrophoresis was observed with ultraviolet (UV) on UV illuminator long live™ Filter spectroline<sup>R</sup> was used to observe fragment visualization with electrophoresis and photographed with a digital camera.

#### Statistical analysis

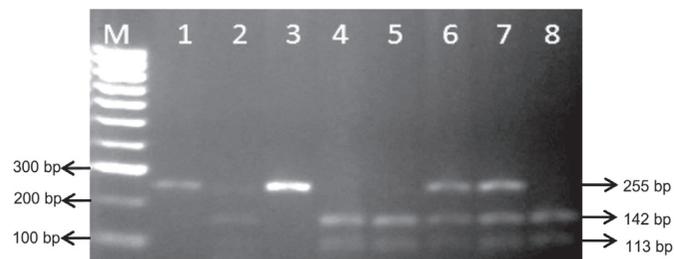
Genotypic polymorphisms distribution and allele frequencies were tested using Thesias 3.0 software program with genetic packages, and continued with SPSS version 21 at p=0.05. Chi-square, Mann-Whitney test, Spearman's correlation (p), t-independent, and two-tailed tests were used to analyze the data.

## RESULT

### *ERα* gene polymorphism rs9340799 A/G, *ERα* gene rs2234693 T/C and *ERβ* gene rs4986938 G/A

The PCR products of *ERα* genes intron 1 rs9340799 restricted from XbaI enzyme showed A/G nucleotide changes (Figure 1), resulting in a DNA band size of 255 bp for homozygous mutant/GG, two DNA bands with the size of 142 bp and 113 bp for homozygous wild-type/AA, and three DNA band with the sizes of 255 bp, 142 bp, and 113 bp for heterozygous/GA.

The *ERα* intron 1 rs2234693 gene PCR products restricted by PvuII showed T/C nucleotide changes (Figure 2), generating a DNA band size of 255 bp for homozygous wild-type/TT, two



**Figure 1.** Polymerase chain reaction-restriction fragment length polymorphism results of gene intron 1 rs 9340799 *ERα* using XbaI. Marker (M), well no: 1 and 3 are the homozygous mutant (GG); well no: 2, 6, and 7 are the heterozygous (AG); and well no: 4 and 5 are the homozygous wild type (AA), ER: Estrogen receptor

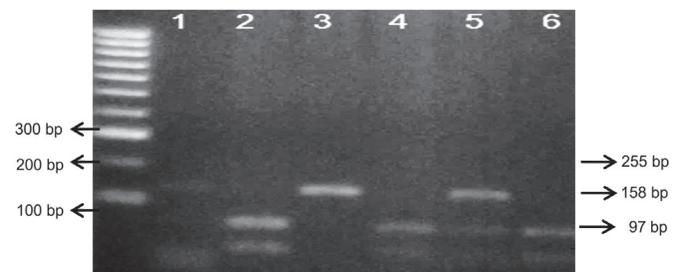
DNA bands with the size of 158 bp and 97 bp for homozygous mutant/CC, and three DNA bands with the size of 255 bp, 158 bp, and 97 bp for heterozygous/TC.

The *ERβ* exon 8 rs4986938 gene PCR products that were cleavage with AluI showed G/A nucleotide changes (Figure 3), which resulted in a DNA band size of 293 bp for homozygous wild-type/AA, two DNA band sizes of 200 bp and 92 bp for homozygous mutant/GG, and three DNA band sizes of 293 bp, 200 bp, and 92 bp for heterozygous/AG.

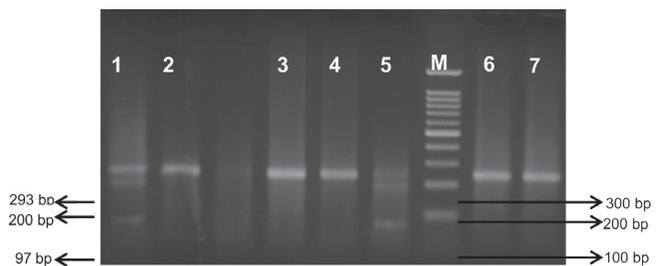
### *Genotype distribution and allele frequency ERα gene rs9340799 A/G, ERα gene rs2234693 T/C and ERβ gene rs4986938 G/A of the case and control groups*

Table 1 shows the genotype comparison distribution and allele frequency of *ERα* gene rs9340799 with XbaI. It shows that GG genotypes (normal) and heterozygous genotype GA were higher in the endometriosis case group than the control, whereas the AA genotype (wild type) was lower in the endometriosis case group than the control. G allele frequency was higher in the endometriosis case group than controls, whereas the frequency of allele A was lower in the endometriosis case group than in control. The Pearson chi-square test results showed genotype frequencies in the case and control groups were not significant. In contrast, the allele frequencies in endometriosis cases and control groups showed no significant differences with p=0.012, and the control group is G allele (mutant).

Table 2 displays the genotype comparison distribution and allele frequencies of *ERα* gene with PvuII. Table 2 shows



**Figure 2.** Polymerase chain reaction-restriction fragment length polymorphism of gene intron 1 rs 2234693 *ERα* using PvuII. Marker (M), well no: 3 is the wild-type genotype (TT); well no: 2, 4, and 6 are the homozygous mutant genotype (CC); and well no: 1 and 5 are the heterozygous genotype (TC), ER: Estrogen receptor



**Figure 3.** Polymerase chain reaction-restriction fragment length polymorphism of *ERβ* exon 8 rs4986938 using AluI. Marker (M), well no: 1 and 5 are the heterozygous genotypes (GA); well no: 2, 3, 4, 6, and 7 are the wild-type homozygous genotype (GG). This image does include the mutant genotype (AA), ER: Estrogen receptor

that the normal genotype (TT) and heterozygous genotype (CT) were lower in the case group than in the control group. Meanwhile, homozygous mutant genotype (CC) was higher in the case group than in the control. T allele frequency in the case group was lower than in the control group, whereas the C allele was higher in the case group compared with the control group. The Pearson chi-square test results demonstrated that the genotype distribution in the case and control groups showed no significant difference ( $p=0.422$ ). Likewise, T and C allele frequency also showed no significant difference ( $p=0.305$ ).

Table 3 shows the genotype comparison distribution and allele frequency of *ER $\beta$*  with restriction AluI. Table 3 shows that the normal genotype (GG) was lower in the case group than in the control, and heterozygous genotype (GA) was higher in the case group than in the control group. In contrast, homozygous mutant genotype (AA) was not found in the case group, but one was found in control. G allele frequency in the case group was lower compared with the control group, whereas the A allele frequency was higher in the endometriosis case group than in the control group.

Pearson chi-square test showed the genotype distribution and G and A allele frequency in the case and control groups were significantly different ( $p=0.015$  and  $p=0.034$ , respectively).

## DISCUSSION

This experiment revealed the proportion of genotype GA in endometriosis was more dominant than the other genotypes. This study revealed that the genotype frequencies in the case and control groups showed no difference. The previous study reported that *ER $\alpha$*  gene rs9340799 amplification changes in nucleotides A to G using XbaI restriction in region intron 1.<sup>11</sup> Study from NCBI showed that individuals with genotype AA normal have a lower risk of cases of endometriosis. In contrast, individuals with genotype GG are ten times higher risk of endometriosis.

Paskulin et al.<sup>11</sup> reported that the amplification of the *ER $\alpha$*  gene region intron 1 SNP rs2234693 T nucleotide changes into C using the restriction PvuII. The second difference allele frequency in both groups (case and control) was conducted by chi-square test. It can be concluded that the genotype frequency in the case and control groups were not different, and statistical tests indicated no difference in allele frequency between the two groups with  $p=0.422$  ( $p>0.05$ ). Hardy-Weinberg balance test also showed no significant difference with  $p>0.05$ . Genotype frequencies did not display any difference between the case and control groups. Ayvaz et al.<sup>13</sup> found that *ER $\alpha$*  gene rs2234693

**Table 1. Genotype and allele frequency of *ER $\alpha$*  rs9340799 gene with XbaI restriction in intron 1 A/G nucleotide change in the case and control groups**

	Genotype			$X^2$	p	Allele frequency		$X^2$	p	OR (CI: 95%)
	AA	AG	GG			A	G			
Control	32 (42.1%)	28 (36.8%)	16 (21.1%)	-	-	92 (60.5%)	60 (39.5%)	-	-	-
Endometriosis case	22 (26.5%)	33 (39.8%)	28 (33.7%)	5.236	0.073	77 (46.4%)	89 (53.6%)	6.372	0.012	2.54
Total	54	61	44	-	-	167	151	-	-	-

ER: Estrogen receptor, OR: Odds ratio, CI: Confidence interval

Note: The normality test of the *ER $\alpha$*  (XbaI) genotype distribution in endometriosis and control groups showed that the data were not normally distributed ( $p<0.05$ ), followed by the chi-square test which showed that the distribution of genotypes in endometriosis and control groups is not significantly different ( $p>0.05$ )

**Table 2. Genotype and allele frequency of *ER $\alpha$*  rs2234693 gene with PvuII restriction in intron 1 T/C nucleotide change in the case and control groups**

	Genotype			$X^2$	p	Allele frequency		$X^2$	p
	TT	TC	CC			C	T		
Control	18 (23.7%)	46 (60.5%)	12 (15.8%)	-	-	70 (46.1%)	82 (53.9%)	-	-
Endometriosis case	17 (20.5%)	46 (55.4%)	20 (24.1%)	1.724	0.422	86 (51.8%)	80 (48.2%)	1.051	0.305
Total	35	92	32	-	-	156	162	-	-

ER: Estrogen receptor

Note: The normality test of *ER $\alpha$*  (PvuII) genotype distribution of endometriosis and control groups were not normally distributed ( $p<0.05$ ) followed by chi-square test which showed genotype frequency was not significantly different with  $p=0.422$  ( $p>0.05$ ), and allele frequency was also not significantly different with  $p=0.305$  ( $p>0.05$ )

**Table 3. Genotype and allele frequency of *ER $\beta$*  rs4986938 gene with AluI restriction in exon 8 G/A nucleotide change in the case and control groups**

	Genotype			$X^2$	p	Allele frequency		$X^2$	p	OR (CI: 95%)
	GG	GA	AA			G	A			
Control	67 (88.2%)	8 (10.5%)	1 (1.3%)	-	-	142 (93.4%)	10 (6.6%)	-	-	-
Endometriosis case	60 (72.3%)	23 (27.7%)	0 (0.0%)	8.35	0.015-0.31	143 (86.1%)	23 (13.9%)	4.517	0.034	0.44
Total	127	31	1	-	-	287	31	-	-	-

ER: Estrogen receptor, OR: Odds ratio, CI: Confidence interval

Note: The normality test of *ER $\beta$*  (AluI) genotype distribution of endometriosis and control groups were not normally distributed ( $p<0.05$ ) followed by chi-square test which showed genotype frequency was significantly different ( $p<0.05$ ) as well as the frequency allele was significantly different  $p=0.034$  ( $p<0.05$ )

with PvuII was associated with a decreased male infertility risk, a change in ER polymorphism function, and, in general, is not comprehensible.

The amplification of the *ERβ* gene region of exon 8 rs4986938 nucleotide changes G/A with AluI restriction was done by Paskulin et al.<sup>11</sup> Statistical test results indicated a significant difference in the genotype frequency of *ERβ* in the case and control groups, with  $p=0.015$  ( $p<0.05$ ).

Allele distribution in *ERβ* showed no significant difference between the normal allele G and mutant allele A in the case group compared with the control group, which means that normal allele G can reduce endometriosis risk 0.44 times. The distribution of normal allele G seemed to dominate than other alleles, which is similar to a study conducted by Zulli et al.<sup>14</sup> It can be concluded that the presence of a significant difference in genotype frequency and distribution of alleles, the gene polymorphism *ERβ* with rs4986938 region of exon 8 could contribute to endometriosis risk due to the mutations in this area can affect the changes of amino acid compositions, and may affect the stop codon shift. The transcription keeps working, which causes the increased gene expression; and possible mutations via deletions, which may cause frameshift errors.<sup>15</sup>

## CONCLUSION

Polymorphism genotyping, gene allele *ERβ* rs4986938 G/A and allele frequencies of *ERα* gene rs9340799 A/G may contribute to the occurrence of endometriosis risk.

## ACKNOWLEDGMENTS

The authors thank The Ministry of Research, Technology, and Higher Education, Republic of Indonesia (Kemenristekdikti RI) for financial support; Department Biology, the Faculty of Mathematics and Natural Science, Universitas Indonesia; and Department of Medical Biology, Faculty of Medicine, Universitas Indonesia for research facilities.

*Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.*

## REFERENCES

- Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril*. 2012;98:511-519.
- Baldi A, Campioni M, Signorile PG. Endometriosis: pathogenesis, diagnosis, therapy and association with cancer (review). *Oncol Rep*. 2008;19:843-846.
- Hendarto H. Profil TNF-, GDF-9 dan hyaluronan pada gangguan folikulogenesis sebagai gambaran penurunan kualitas oosit pasien infertil dengan endometriosis. Dissertation. 2007. Universitas Airlangga. Surabaya. Indonesia. <http://repository.unair.ac.id/31983/>
- Wiweko B, Puspita CG, Tjandrawinata R, Situmorang H, Harzi AK, Pratama G, Sumapraja K, Natadisastra M, Hestiantoro A. The effectiveness of *Phalleria macrocarpa* bioactive fraction in alleviating endometriosis and/or adenomyosis related pain. *eJ Ked Indonesia* 2015;3:51-56.
- Tanbo T, Fedorcsak P. Endometriosis-associated infertility: aspects of pathophysiological mechanisms and treatment options. *Acta Obstet Gynecol Scand*. 2017;96:659-667.
- Siva AB, Srivastava P, Shivaji S. Understanding the pathogenesis of endometriosis through proteomics: recent advances and future prospects. *Proteomics Clin Appl*. 2014;8:86-98.
- Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol*. 2014;10:261-275.
- Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med*. 2013;19:197-209.
- Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev*. 2007;87:905-931.
- Bulun SE, Cheng YH, Pavone ME, Xue Q, Attar E, Trukhacheva E, Tokunaga H, Utsunomiya H, Yin P, Luo X, Lin Z, Imir G, Thung S, Su EJ, Kim JJ. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin Reprod Med*. 2010;28:36-43.
- Paskulin DD, Cunha-Filho JS, Paskulin LD, Souza CA, Ashton-Prolla P. ESR1 rs9340799 is associated with endometriosis-related infertility and *in vitro* fertilization failure. *Dis Markers*. 2013;35:907-913.
- Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, Tokunaga H, Su EJ. Role of estrogen receptor-β in endometriosis. *Semin Reprod Med*. 2012;30:39-45.
- Ayvaz OU, Ekmekçi A, Baltacı V, Onen HI, Unsal E. Evaluation of *in vitro* fertilization parameters and estrogen receptor alpha gene polymorphisms for women with unexplained infertility. *J Assist Reprod Genet*. 2009;26:503-510.
- Zulli K, Bianco B, Mafra FA, Teles JS, Christofolini DM, Barbosa CP. Polymorphism of the estrogen receptor β gene is related to infertility and infertility-associated endometriosis. *Arq Bras Endocrinol Metabol*. 2010;54:567-571.
- Bebenek K, Garcia-Diaz M, Blanco L, Kunzel TA. The frameshift infidelity of human DNA polymerase lambda. Implications for function. *J Biol Chem*. 2003;278:34685-34690.