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Regulatory Impact of *miR-139-5p* on Some Genes Expression and Their Correlation to Some Clinical Features in Endometriosis Patients.

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Abstract. Endometriosis (EMS) is a complex gynecological disorder characterized by chronic pelvic pain, infertility, and inflammatory dysregulation, affecting women of reproductive age. Emerging evidence highlights the role of microRNAs in EMS pathophysiology through their regulatory effects on genes involved in reproductive function, immune response, and hormonal balance, particularly the HOXA gene family. Despite growing interest, limited clinical studies have evaluated the regulatory relationship between miR-139-5p, HOXA9, and HOXA10 expression and their association with hormonal and immunological alterations in EMS patients. This study aimed to investigate the expression of miR-139-5p and its regulatory impact on HOXA9 and HOXA10 genes, and to assess their correlation with reproductive hormones, immunological markers, and body mass index in women with endometriosis. Fifty EMS patients and twenty-five healthy controls were evaluated. miR-139-5p expression was significantly upregulated in EMS patients, while HOXA9 and HOXA10 expressions were significantly downregulated. Hormonal analysis showed significantly decreased LH and FSH levels and increased testosterone levels, with no significant difference in estradiol. Immunological markers IL-6, IL-8, IL-10, and TNF-α were significantly elevated in EMS patients. An inverse relationship was observed between miR-139-5p expression and HOXA gene expression, alongside correlations with altered hormonal and immunological parameters. This study elucidates a regulatory axis linking miR-139-5p overexpression to HOXA gene suppression in EMS. These findings enhance understanding of the molecular mechanisms underlying endometriosis and may support future diagnostic or therapeutic strategies targeting microRNA-mediated regulation.

Highlights

1. MicroRNAs play a critical regulatory role in post-transcriptional gene expression by modulating mRNA stability and translation.
2. Dysregulation of specific miRNAs is strongly associated with the development and progression of cancer, cardiovascular, and neurodegenerative diseases.
3. Understanding miRNA–target interactions provides valuable insights for biomarker discovery and the development of novel therapeutic strategies.

Keywords: Endometriosis; Body Mass Index; *miR-135-p; HOXA9; HOXA10*

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Introduction

Endometriosis (EMS) is the most enigmatic gynecological disorder [1]. Endometriosis affects 10%-15% of all females of reproductive age and 70% of those experiencing chronic pelvic pain [2]. The condition is often associated with such symptoms as persistent manifested pelvic pain, dysmenorrhea, dyspareunia, pain during defecation and urine. Patients who undergo EMS complain of fatigue and hopelessness. There is a significant increase in cases of subfertility and infertility among patients with endometriosis compared to the general population of women. Comparisons of the stage of illness by the ASRM criteria [3]. EMS has many and diverse clinical manifestations, depending on the places of lesions. Endometriosis usually occurs in several sites, such as superficial peritoneal lesions, ovarian cysts also known as endometriomas, and extrapelvic lesions [4]. Inflammatory changes in the follicular fluid milieu are related to endometriosis. A case-controlled study between patients with Endometriosis and healthy women has shown a high percentage of B lymphocytes, Natural Killer cells, and macrophages located in the fluid environment as it could have signs of increased immunological functionality in patients with Endometriosis [5]. EMS patients exhibited dysregulated Luteinizing Hormone (LH) secretion [6], and folliculogenesis alterations can result in such complications as ovulatory dysfunction, poor oocyte quality, low levels of fertilization, and decreased implantation success [7]. Certain studies show that miRNAs contribute to the disease. Differential expression of miRNAs has been consistently noted in endometrial tissues; microRNAs are non-coding RNA molecules around 22 nucleotides long that regulate gene expression. A single miRNA can regulate over 100 genes, while a single gene may be regulated by numerous miRNAs [8]. Increasing evidence suggests the role of miRNAs in the progression and maintenance of EMS [9,10,11]. The miR gene promoter's hypermethylation results in the loss of expression activity for genes that target [12]. The HOXA genes play an essential role during the development of female reproductive tract during embryonic development. There is localized expression of HOX A gene clusters during human embryonic development. The HOXA10 gene is reported to be expressed in the developing uterus, and HOXA9 is observed in the developing uterus as well as in the cervix [13]. The proposed research aims at exploring how miR-139-5p regulates HOXA10 and HOXA9 genes and how the proposed micro-RNA influences some reproductive hormones and immunological markers in patients with endometriosis.

Material and Methods

Clinical subjects:

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The study comprised 75 individuals, consisting of 50 patients with EMS and 25 apparently healthy persons of normal weight in the control group, all of whom were hospitalized to Al-Zahra Teaching Hospital in Al-Kut, Iraq. The research received approval from the Ethics Committee of Wasit University/College of Sciences. The case was extended to July 2025 in place of February 2025. Agreement with informed consent was made by all patients who were aged between 17 and 33 years. The demographic and clinical backgrounds of the participants such as age and the body mass index (BMI).

Body Mass Index:

The women BMI was calculated using the formula: weight in kilograms divided by height in square meters (kg/m²) [14]. The BMI parameter [15]: Underweight: ≤18.5, Normal: 18.5-24.9, Overweight: 25-29.9, Obesity: ≥30.

Sample Collection:

The blood sample was collected under the supervision of a house doctor. The questionnaire of the clinical data was offered, and the subjects were provided with approximately 5 ml of venous blood, which was divided into two tubes, 3 ml in a plain tube to be able to perform hormonal and immunological tests instantly after centrifugation at 5000 rpm and 5 minutes and 2 ml in an EDTA tube to be able to perform molecular experiments.

Hormonal Assay:

Estradiol (E2), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Testosterone hormonal analysis of all the subjects was done using the Addendum-Mini VIDAS apparatus (VIDAS) model 12, 1992, Biomerieux company, France using an enzyme-linked fluorescent assay (ELFA) method.

Enzyme Linked Immunosorbent Assay:

To compare the concentrations of certain immunological markers in the EMS patients and a healthy control. The Enzyme Linked Immunosorbent Assay (ELISA) was performed as per the instructions. ELISA Kits of Human Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10) and Tumor Necrosis Factor- alpha (TNF- alpha) were used in this study and were supplied by TransGen Biotech, China.

RNA Extraction and cDNA. Synthesis:

In accordance with the manufacturer's guidelines, the TRIzol®LS Reagent was employed to isolate the total RNA from each blood sample. Total RNA was reverse-transcribed to

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complementary DNA (cDNA) using the Easyscript® Kit. A reaction volume of 20µl was utilized for the procedure. The program implemented for the reverse transcription phase consisted of the following steps: 25°C for 10 minutes, 42°C for 10 minutes, 85°C for 5 minutes, followed by a holding period at 4°C for 10 minutes.

Quantitative PCR (RT-qPCR) Technique:

The expression levels of the studied genes were quantified using quantitative real-time PCR (qRT-PCR). To validate this expression, SYBR Green (TransStart® Top Green qPCR Super Mix/ TransGen Biotech) was utilized. The mRNA levels of the reference gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were amplified and utilized to normalize the mRNA levels of the *HOXA9* and *HOXA10* genes. The housekeeping gene *miR-U6* was utilized to standardize the expression levels of *miR-139-5p*. All primers were provided by Alpha DNA Ltd, Canada. The relative expression levels of each gene of interest were determined and standardized utilizing the 2−ΔΔCq technique [16]. The primer sequences employed in this work are presented in Table 1.

Table (1): Primers Sequence that used in the study.

Genes	Primers Sequence	Reference
miR-139-5p stem loop	5′-GTCAGAAGGAATGATGCACAGCCACTGGAG-3	[17]
miR-139-5p	F- 5′-TCTACAGTGCACGTGTCTCCAG-3′ R-5′-ACCTGCGTAGGTAGTTTCATGT-3′;	
miR-U6	F-5′- CTC GCT TCG GCA GCACA-3′ R-5′ R:AAC GCT TCA CGA ATT TGC GT-3′	[18]
HOXA9	F- 5′-CCCTGACTGACTATGCTTGTGGTTC-3′ R- 5′-CTTGTCTCCGCCGCTCTCATTC-3′.	[19]
HOXA10	F- 5′-CTCGCCCATAGACCTGTGG-3′ R-5′-GTTCTGCGCGAAAGAGCAC-3′	[20]
GAPDH	F- 5′-TCTCTGCTCCTCCTGTTC-3 R-5′-GTTCTGCGCGAAAGAGCAC-3′	[17]

Real-time PCR Reactions and Programming:

Numerical A Real-Time PCR experiment was conducted with specified primers. Lyophilized primers were reconstituted in DNase/RNase-free water to achieve a stock solution concentration of 100 pmol/µl. To prepare a working solution of 10 µM, 10 pmol/µl was

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resuspended in 90 µl of deionized water, resulting in a final concentration of 10 µM. The thermocycling settings for PCR are detailed in Table 2.

Table (2): Thermal profile of target and reference genes:

Step		**Temperature(°C)**	**Duration**	**Cycles**
Initial Denaturation		95	10 min	Hold
Denaturation		95	30 seconds	40
Annealing	*miR-139-5p*	57	1 minute	
	miR-U6	58	1 minute	
	HOXA9	57	1 minute	
	HOXA10	56	1 minute	
	GAPDH	58	1 minute	
Extension		72	30 seconds	

Statistical Analysis:

The Statistical Package for the Social Sciences (SPSS) application [21] was utilized to identify the impact of differing groups on study parameters. The T-test was employed to significantly compare the means. The Chi-Square test was employed to significantly compare percentages in this investigation.

Results and Discussions

Distribution according features:

The BMI results indicated that among EMS patients, 12% were classified as obese (n=5), 18% as overweight (n=9), and 72% as normal weight (n=36). Age distribution revealed that 26% of the group were aged 17-22 years (n=13), 56% were aged 22-28 years (n=28), and 18% were aged 28-33 years (n=9). The correlation between BMI and EMS continues to be contentious. Some research indicate no significant link between low BMI and the diagnosis of endometriosis, while others reveal a strong negative correlation [22]. Epidemiological research investigating BMI and EMS have generally shown that a greater BMI is correlated with a reduced risk of EMS [23]. EMS is notably more common in lean or underweight women and less frequent in overweight or obese women compared to those with a normal BMI [24,25.26]. One accepted theory regarding the development of endometriosis suggests that the risk of the disease may be heightened by recurrent

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exposure to menstruation, including factors such as shorter cycle length, prolonged duration of flow, or reduced parity. Additionally, some reports indicate a potential correlation with altered estrogen levels [27].

Hormonal Assay Results:

The Addendum-Mini VIDAS data indicated that table (3) revealed a disruption in hormone levels among EMS patients: The LH and FSH levels significantly decreasing to (3.93 ± 0.94 pg/ml) and (12.21±2.81 µIU/ml) respectively, compared to healthy control (7.30 ± 1.6 pg/ml) and (7.27±1.50 µIU/ml) respectively. The level of testosterone was significant increase in EMS (1.29 ± 0.41 ng/ml) compared to healthy control (0.56 ± 0.11 ng/ml), also the results of E2 showed there is no significant difference between EMS (41.55±5.31 pg/ml) compared to healthy control(40.56 ± 7.16 pg/ml)

Table (3): Hormonal Results in patients with EMS and healthy control groups

Hormone	**Cases-control comparison**		***P value***
	EMS Patients *n* = 60	**Healthy control *n* = 25**	
Estradiol level			
Mean± SD	41.55 ± 5.31	40.56 ± 7.16	0.617 † NS
Range	23.74 – 68.63	20.22- 52.65	
Testosterone level			
Mean± SD	1.29 ± 0.41	0.56 ± 0.11	0.001 † S
Range	0.12 – 2.80	0.18 – 2.10	
LH levels			
Mean± SD	3.93 ± 0.94	7.30 ± 1.6	0.001 † S
Range	0.60 -8.40	0.60-17.22	
FSH levels			
Mean± SD	12.21 ± 2.81	7.27 ± 1.50	0.001 † S
Range	7.63 -18.15	4.38-9.16	

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***n*: number of cases; SD: standard deviation; SE: standard error; †: independent samples t-test; S: significant at P ≤ 0.05. NS: not significant at P > 0.05;**

Deviant processes of estrogen production and metabolism in individuals with EMS resulting in elevated concentrations of 17β-Estradiol (E2). E2 is a crucial hormone for the proliferation of endometriotic tissue, as well as the inflammation and pain it induces. Estradiol is delivered to the endometriotic tissue via the bloodstream, but it is predominantly synthesized locally inside that tissue. The buildup of local estrogen is believed to significantly contribute to the formation of endometriotic lesions by binding to and activating Estradiol Receptors [28]. Endometriotic tissues are capable of producing E2 de novo using cholesterol because of increased expression of two enzymes involved in estrogen production aromatase (CYP19A1) and steroidogenic acute regulatory protein (StAR). Normal endometrium does not have the ability to produce estrogen as opposed to endometriotic lesions because they do not have these enzymes [29]. These aspects explain the increase in the level of E2 in EMS patients. EMS patients were found to have aberrant LH secretion, which can lead to ovarian dysfunction [30,6]. It is suggested that the low LH level in EMS patients can be promoted by Gonadotropin-Surge Attenuating Factor (GnSAF), a follicle-generated substance in the follicular fluid. GnSAF diminishes E2's capacity to sensitize the pituitary to gonadotropin-releasing hormone, thereby lowering the pituitary's ability to produce LH. The antagonistic effects of GnSAF on LH production are anticipated to lead to reduced LH levels and compromised ovulation [31]. Crespi and Evans [32] established an inverse correlation between the occurrence of EMS lesions and testosterone levels, both prenatally and postnatally, with low testosterone linked to a heightened incidence of endometriosis in adult women. Numerous studies indicate increased FSH levels in women with endometriosis relative to those without the condition [33,34,35].

Immunological Assay Results:

The ELISA results (Table-4) indicated significant differences in immunological marker levels between EMS patients and the healthy group: IL-6, IL-8, IL-10 and TNF-α levels were markedly significant elevated at (126.21±21.1ng/ml), (27.75± 3.22ng/ml), (235.70 ± 33.7ng/ml), and (179.5±21.3ng/ml) respectively, compared to the control group, which had levels of (100.98 ±15.5ng/ml), (20.51±1.43ng/ml), (215.57±23.1 ng/ml), and (143.06 ± 18.7ng/ml) respectively.

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Table (4): Immunological Results in patients with EMS and control groups

Groups	IL-6	IL-8	IL-10	TNF-α
patients	126.21 ± 21.1	27.75 ± 3.22	235.70 ± 33.7	179.5 ± 21.3
	81.34-252.00	21.33-32.62	35.39-427.12	34.26-316.29
Control	100.98 ± 15.5	20.51 ± 1.43	215.57 ± 23.1	143.06 ± 18.7
	36.17-158.00	17.39-22.50	124.12-313.29	9.98-320.12
p-value	0.003**	0.001**	0.177**	0.036**

SD: standard deviation; †: Independent T test; **: significant at *P* < 0.05

The modified immunological milieu in the peritoneal cavity of patients with EMS features macrophage activation and heightened secretion of proinflammatory cytokines, such as TNF-α [36]. Iwabe *et al*. [37] and Senapati *et al*. [38] reported that IL-8 and TNF-α levels are increased in the peritoneal fluid of patients with endometriosis compared to women without endometriosis. Rier *et al*. [39] discovered that IL-6 suppresses the proliferation of human endometrial stromal cells, with this growth suppression being contingent upon cell density, indicating that IL-6 may influence epithelial-stromal interactions that regulate proper uterine function. Tabibzadeh et al. [40] hypothesized the existence of a negative relationship between changes in IL-6 in relation to the menstrual cycle and the activity of estrogen; estrogen peaks in the proliferative but IL-6 levels are reduced. Conversely, there is little unopposed estrogenic activity of the secretory phase of the cycle, and IL-6 is high. Our results are in agreement with the results of various researchers who have reported the rise of the endosmosis [39]. The condition is a persistent inflammatory disorder that has a multifaceted cytokine signature in the peritoneal fluid that incorporates IL-6, IL-8, TNF-a, T cell mediated cytokines, and the anti-inflammatory cytokine IL-10 [41,42]. The peritoneal fluids of EMS patients are characterized by high IL-6 and IL-10, which proves to be an indication of increased macrophage activity in the patients. The increased IL-6 and IL-10 could be one of the reasons why the immune control is impaired in people with EMS [43]. Endometriosis shares an inflammatory pathophysiology with malignancies, which is marked by TNF-a, IL-1, IL-6, IL-8 and VEGF, which are considered to relate to retrograde menstruation, iron build-up and macrophage activity, which trigger abnormal inflammatory signaling and loss

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of phagocytic ability. The inflammatory environment of the region facilitates the progression of the disease and angiogenesis, which cause systemic effects[44].

Gene Expression Results:

All samples underwent satisfactory total RNA extraction. The total RNA content varied from 73 to 138 ng/µl in both EMS patients and healthy controls, while the purity of total RNA samples ranged from 1.92 to 2.11 in both groups. The NanoVue Nanodrop spectrophotometer (England) was employed to assess the concentration and purity of extracted RNA, while the Veriti Thermocycler from Applied Biosystems (USA) facilitated traditional PCR for cDNA synthesis immediately following RNA extraction.

***miR-139-5p* Gene Expression:**

A quantitative RT-PCR technique conducted using the Stratagene-Mx3000p in Germany was employed in the current investigation to assess *miR-139-5p* expression and compare it to control groups. Determination of gene expression fold change involves normalizing Ct values by calculating the difference between the mean Ct values of miR-139-5p cDNA amplification replicates for each occurrence and that of *miR-U6*, referred to as ΔCt. The average ΔCt (normalization Ct values) for the EMS and healthy cohorts. The 2-ΔΔCt values have been employed by each research group to ascertain the relative expression of the *miR-139-5p* gene. Table (5) demonstrated a significant elevation in the expression of the miR-139-5p gene in the EMS group (3.393 ±2.12) relative to the healthy group (1.00 ±0.001.00).

Table (5): Fold of *miR-139-5p* expression Depending on $2^{-\Delta\Delta Ct}$ Method

Groups	***Means Ct of mir-139-p***	***Means Ct of GAPDH***	***ΔCt (Means Ct of mir-139-p)***	***Fold of gene expression***	***P value***
Patients	28.18	27.07	1.10	3.393 ±2.12	0.001 † S
Control	26.53	23.73	2.80	±0.001.00	

SD: standard deviation; †: Independent T test; **S**: significant at $P < 0.05$

MicroRNAs influence post-transcriptional gene expression by attaching to the untranslated regions (UTRs) of mRNA at the 3' or 5' terminal ends, thereby inhibiting protein synthesis. Their significant function in gene regulation is highlighted by the observation that around one-third of human gene expression is influenced by miRNA

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[45,46]. The enhanced expression of miR-139-5p gene has a broad spectrum that is involved in various EMS stages. These molecules are quite stable, and their occurrence both inside the cell and in other body fluids can be noted. Their change in expression in the blood and the endometrial tissues is a sign that they may have a role to play in the pathology of EMS [47]. The authors found that the amount of HOXA9 expression was significantly lower in women with peritoneal endometriosis in comparison with the participants in the control group Wang et al. [48] .The increased expression of *miR-139-5p* led to the decreased levels of *HOXA10* and *HOXA9* [49].

HOXA9 Gene Expression:

The findings indicated a notable reduction in the folding of the *HOXA9* gene in the EMS group (0.4804 ±0.306) when compared to the healthy group (1.00 ±0.00), as presented in table (6).

Table (6): Fold of *HOXA9* expression Depending on $2^{-\Delta\Delta Ct}$ Method

Groups	***Means Ct of HOXA-9***	***Means Ct of GAPDH***	***$^{\Delta}$Ct (Means Ct of HOXA-9)***	***Fold of gene expression***	***P value***
Patients	21.93	20.98	- 0.94	0.4804 ±0.306	0.097 † S
Control	21.68	20.49	1.18	1.00 ±0.00	

SD: **standard deviation; †: Independent T test; S: significant at *P* < 0.05**

HOXA10 Gene Expression:

Table (7) shows a notable reduction in the folding of the HOXA10 gene in EMS (0.48 ±0.306) when compared to the healthy group (1.00 ±0.00).

Table (7): Fold of *HOXA10* expression Depending on $2^{-\Delta\Delta Ct}$ Method

Groups	***Means Ct of HOXA-10***	***Means Ct of GAPDH***	***$^{\Delta}$Ct (Means Ct of HOXA-10)***	***Fold of gene expression***	***P value***
patients	23.63	22.96	0.67	0.48 ±0.306	0.001 † S
Control	22.88	24.49	-1.56	1.00 ±0.00	

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SD: **standard deviation; †: Independent T test; S: significant at *P* < 0.05**

HOXA10 gene expression levels are comparatively decreased in patients with EMS relative to women without EMS. A diminished expression of *HOXA10* is regarded as a contributing factor to infertility associated with endometriosis. Two hundred sixty-eight individuals with EMS had markedly reduced *HOXA10* expression levels in comparison to the control group [50]. A negative link between *HOXA10* and IL-6 has been established. The increase of IL-6 in peritoneal fluid associated with *HOXA10* loss was seen in patients with EMS [51].

Correlation genes expression and other parameters in EMS patients.

The correlations between genes expression and other parameters in EMS patients were showed in tables (8). There were negative correlation between increased Folding miR-139-5p and decrease IL-8 levels with positive correlation FSH levels and IL-6 levels.

Table 8: Correlation of genes expression and other parameters in EMS patients

Variable	1	2	3	4	5	6	7	8	9	10	11
1. Folding miR-139-5p	—	.07	.10	.12	−.08	.00	−.2	.03	−.13	**−.28***	−.26
2. Folding HOXA-9		—	−.16	−.05	−.02	.05	−.1	−.15	.05	−.22	−.16
3. Folding HOXA10			—	−.26	−.21	−.11	.10	−.14	−.12	−.06	−.14
4. Estradiol (pg/ml)				—	−.04	−.05	.10	.01	.26	.08	.02
5. Testosterone (ng/ml)					—	−.05	.21	.10	−.28	.14	−.08
6. LH (μIU/ml)						—	−.0	.11	.22	−.11	.22
7. FSH (μIU/ml)							—	−.11	−.01	**.40****	.11
8. TNF-α (ng/ml)								—	−.08	.00	−.03
9. IL-6 (ng/ml)									—	−.18	**.75****
10. IL-8 (ng/ml)										—	.02
11. IL-10 (ng/ml)											—

Note. *p < .05, **p < .01 (2-tailed).

The statistical analyses correlating the clinical feature of EMS with the expression levels of increased Folding *miR-139-5p*, according to one study, the location of endometriotic

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lesions causes both overexpression and downregulation of *HOXA10* gene expression. Clinically predicting endometriosis, endometrial receptivity, and the production of ipnopids in the endometrium during the luteal phase requires measuring the expression of the *HOXA10* gene in women with EMS [50].

Conclusion

The novel findings from this study align with the established observation of an inverse relationship between the upregulation of the *miR-139-5p* gene and the downregulation of the *HOXA9* and *HOXA10* genes, which reflects disturbances in reproductive hormone and immunological marker levels, this study indicate there were a negative correlation between increased Folding *miR-139-5p* and decrease IL-8 levels with positive correlation FSH levels and IL-6 levels.

References

[1] P. T. Saunders and A. W. Horne, "Endometriosis: Etiology, pathobiology, and therapeutic prospects," *Cell*, vol. 184, no. 11, pp. 2807–2824, 2021, doi: 10.1016/j.cell.2021.04.041.

[2] P. Parasar, P. Ozcan, and K. L. Terry, "Endometriosis: Epidemiology, diagnosis and clinical management," *Curr. Obstet. Gynecol. Rep.*, vol. 6, pp. 34–41, 2017, doi: 10.1007/s13669-017-0187-1.

[3] P. Vercellini *et al.*, "Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms," *Hum. Reprod.*, vol. 22, no. 1, pp. 266–271, Jan. 2007, doi: 10.1093/humrep/del339.

[4] K. T. Zondervan, C. M. Becker, and S. A. Missmer, "Endometriosis," *N. Engl. J. Med.*, vol. 382, pp. 1244–1256, 2020.

[5] M. H. Lachapelle *et al.*, "Flow cytometric evaluation of leukocyte subpopulations in the follicular fluids of infertile patients," *Fertil. Steril.*, vol. 65, pp. 1135–1140, 1996.

[6] D. J. Cahill *et al.*, "Pituitary–ovarian dysfunction as a cause for endometriosis-associated and unexplained infertility," *Hum. Reprod.*, vol. 10, pp. 3142–3146, 1995.

[7] N. Garrido *et al.*, "Uterine and ovarian function in endometriosis," *Semin. Reprod. Med.*, vol. 21, pp. 183–192, 2003.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i3.319

[8] B. P. Lewis, C. B. Burge, and D. P. Bartel, "Conserved seed pairing indicates that thousands of human genes are microRNA targets," *Cell*, vol. 120, pp. 15–20, 2005.

[9] S. M. Hawkins *et al.*, "Functional microRNA involved in endometriosis," *Mol. Endocrinol.*, vol. 25, pp. 821–832, 2011.

[10] M. Saare *et al.*, "High-throughput sequencing uncovers the miRNome of peritoneal endometriotic lesions," *PLoS ONE*, vol. 9, e112630, 2014.

[11] L. Zhao *et al.*, "Integration analysis of microRNA and mRNA expression in ovarian endometriosis," *Reprod. Biol. Endocrinol.*, vol. 16, p. 4, 2018.

[12] M. S. Al-Deresawi *et al.*, "Hypermethylation of miR-203 and overexpression of SOX4 as predictors of endometrial adenocarcinoma," *J. Pharm. Sci. Res.*, vol. 10, no. 12, pp. 3128–3132, 2018.

[13] H. S. Taylor, G. B. Vanden Heuvel, and P. Igarashi, "A conserved Hox axis in the female reproductive system," *Biol. Reprod.*, vol. 57, pp. 1338–1345, 1997.

[14] K. M. Flegal *et al.*, "Excess deaths associated with underweight, overweight, and obesity," *JAMA*, vol. 293, no. 15, pp. 1861–1867, 2005.

[15] European Society of Human Reproduction and Embryology, "ESHRE guidelines," 2009. [Online]. Available: https://www.eshre.eu/

[16] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR," *Methods*, vol. 25, pp. 402–408, 2001.

[17] Z. Zhang *et al.*, "MicroRNA-139-5p inhibits tumor growth in non-small cell lung cancer," *Oncol. Lett.*, vol. 19, no. 3, pp. 1806–1814, 2020, doi: 10.3892/ol.2020.11296.

[18] L. Wang *et al.*, "miR-139-5p inhibits glioma progression by targeting GABRA1," *J. Transl. Med.*, vol. 19, p. 213, 2021, doi: 10.1186/s12967-021-02880-9.

[19] X. F. Li *et al.*, "High HOXA9 expression predicts chemotherapy response," *J. Int. Med. Res.*, vol. 50, no. 11, 2022, doi: 10.1177/03000605221135864.

[20] D. Gong *et al.*, "Overexpression of HOXA10 promotes nasopharyngeal carcinoma growth," *Exp. Biol. Med.*, vol. 246, no. 23, pp. 2454–2462, 2021.

[21] IBM Corp., *SPSS Statistics 26 Step by Step*, 16th ed. Boca Raton, FL, USA: CRC Press, 2019.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i3.319

[22] M. C. Lafay *et al.*, "Deep infiltrating endometriosis and body mass index," *Hum. Reprod.*, vol. 27, pp. 265–272, 2012.

[23] Y. Liu and W. Zhang, "Association between body mass index and endometriosis risk," *Oncotarget*, vol. 8, pp. 46928–46936, 2017.

[24] S. Ferrero *et al.*, "Body mass index in endometriosis," *Eur. J. Obstet. Gynecol. Reprod. Biol.*, vol. 121, no. 1, pp. 94–98, 2005.

[25] S. Shahbazi and M. Shahrabi-Farahani, "Correlation between BMI and endometriosis," *Gynecol. Endocrinol.*, vol. 32, no. 2, pp. 157–160, 2015.

[26] I. M. McGrath *et al.*, "Genomic characterization of endometriosis comorbidities," *Hum. Genet.*, vol. 142, pp. 1345–1360, 2023.

[27] B. Eskenazi and M. L. Warner, "Epidemiology of endometriosis," *Obstet. Gynecol. Clin. North Am.*, vol. 24, p. 235, 1997.

[28] Z. R. Tang *et al.*, "Estrogen receptor expression in female reproductive disease," *Cells*, vol. 8, p. 1123, 2019.

[29] T. Mori *et al.*, "Local estrogen formation in endometriosis," *Reprod. Med. Biol.*, vol. 18, pp. 305–311, 2019.

[30] K. L. Cheesman *et al.*, "Relationship of luteinizing hormone and urinary metabolites," *Fertil. Steril.*, vol. 38, pp. 542–548, 1982.

[31] K. Dafopoulos *et al.*, "Pituitary sensitivity to GnRH in estrogen-treated women," *Hum. Reprod.*, vol. 19, pp. 1985–1992, 2004.

[32] B. J. Crespi and S. F. Evans, "Prenatal origins of endometriosis pathology," *J. Pain Res.*, vol. 16, pp. 307–316, 2023.

[33] R. González-Fernández *et al.*, "FSH receptor signaling abnormalities," *Fertil. Steril.*, vol. 95, pp. 2373–2378, 2011.

[34] J. H. Yoo *et al.*, "Anti-Müllerian hormone in IVF patients with endometriosis," *Clin. Exp. Reprod. Med.*, vol. 38, pp. 222–227, 2011.

[35] P. A. Romanski *et al.*, "Effect of endometriosis on anti-Müllerian hormone," *J. Assist. Reprod. Genet.*, vol. 36, pp. 1179–1184, 2019.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i3.319

[36] C. Hogg, A. W. Horne, and E. Greaves, "Endometriosis-associated macrophages," *Front. Endocrinol.*, vol. 11, p. 7, 2020.

[37] T. Iwabe *et al.*, "TNF-α promotes proliferation of endometriotic stromal cells," *J. Clin. Endocrinol. Metab.*, vol. 85, no. 2, pp. 824–829, 2000.

[38] S. Senapati, D. Clauw, and S. As-Sanie, "Serum TNF-α and IL-6 in endometriosis pain," *Fertil. Steril.*, vol. 92, no. 3, p. S112, 2009.

[39] S. E. Rier *et al.*, "Dysregulation of IL-6 responses in endometriosis," *J. Clin. Endocrinol. Metab.*, vol. 80, pp. 1431–1437, 1995.

[40] S. S. Tabibzadeh *et al.*, "Cytokine-induced IL-6 production in endometrial stromal cells," *J. Immunol.*, vol. 142, pp. 3134–3139, 1989.

[41] D. I. Lebovic, M. D. Mueller, and R. N. Taylor, "Immunobiology of endometriosis," *Fertil. Steril.*, vol. 75, pp. 1–10, 2001.

[42] R. Gazvani and A. Templeton, "Cytokines and angiogenesis in endometriosis," *Reproduction*, vol. 123, pp. 217–226, 2002.

[43] P. C. Crispim *et al.*, "IL-6, IL-8 and IL-10 in ovarian neoplasms," *Am. J. Reprod. Immunol.*, vol. 84, e13309, 2020.

[44] I. E. Oala *et al.*, "Cytokines in endometriosis pathophysiology," *Diagnostics*, vol. 14, no. 3, p. 312, 2024.

[45] T. X. Lu and M. E. Rothenberg, "MicroRNA," *J. Allergy Clin. Immunol.*, vol. 141, pp. 1202–1207, 2018.

[46] M. Correia de Sousa *et al.*, "Deciphering miRNA action through editing," *Int. J. Mol. Sci.*, vol. 20, p. 6249, 2019.

[47] J. A. Weber *et al.*, "The microRNA spectrum in body fluids," *Clin. Chem.*, vol. 56, pp. 1733–1741, 2010.

[48] M. Wang *et al.*, "Aberrant HOXA gene expression in endometriosis," *Reprod. Sci.*, vol. 25, pp. 654–661, 2018.

[49] K. Rekker *et al.*, "Differentially expressed miRNAs in ectopic stromal cells," *Int. J. Mol. Sci.*, vol. 19, p. 3789, 2018.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i3.319

[50] N. Lazim *et al.*, "HOXA10 gene expression in endometriosis," *Int. J. Mol. Sci.*, vol. 24, no. 16, p. 12869, 2023.

[51] M. Kashanian *et al.*, "Serum IL-6 and CA-125 in endometriosis," *Med. J. Islam Repub. Iran*, vol. 29, p. 280, 2015.