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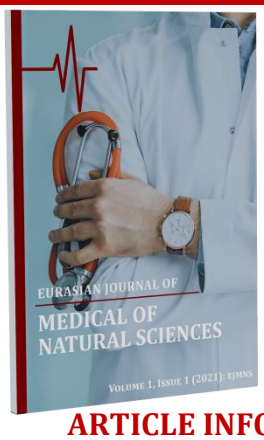

EPIGENETIC MARKERS FOR PREDICTING THE RISK OF DEVELOPMENT OF EMPTY FOLLICLE SYNDROME IN REPRODUCTIVE-AGE WOMEN

Olimova K.J.
Shukurov F.I.
Akhmadzhanova X.Z.
Jurayeva A.J.
Yuldasheva M.A.
Tashkent Medical Academy, Tashkent, Uzbekistan
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Empty follicle syndrome, epigenetic markers, DNA methylation, microRNA, histone modifications, in vitro fertilization, risk prediction.

ABSTRACT

Introduction: *Empty follicle syndrome (EFS) is a rare but serious condition that reduces the chances of successful conception in women undergoing infertility treatment using in vitro fertilization (IVF). Despite the significance of epigenetic mechanisms in regulating reproductive function, their role in the pathogenesis of EFS remains underexplored.* ***Objective:*** *The aim of this study was to identify and analyze epigenetic markers associated with the development of EFS and to assess their prognostic significance.* ***Materials and Methods:*** *A prospective observational study was conducted involving reproductive-age women (18-41 years) with a history of EFS undergoing IVF. The evaluation of epigenetic markers included DNA methylation analysis (bisulfite sequencing methods), microRNA expression (RT-qPCR), and histone modifications (ChIP-sequencing). Data were statistically processed using SPSS software, including Student's t-test, Mann-Whitney test, correlation analysis, and ROC analysis for the development of a prognostic model.* ***Results:*** *Women with EFS showed higher levels of methylation in the FSHR, LHR, and AMH genes, as well as reduced levels of microRNA expression of miR-126 and miR-145. In contrast, there was an increase in the expression of miR-155 and miR-21, as well as a decrease in acetylation of histones H3K9ac and H4K16ac. Correlation analysis revealed negative associations between FSHR methylation and AMH levels, as well as positive associations between miR-126/miR-145 expression and IVF success. The developed multifactorial prognostic model for EFS risk demonstrated high accuracy (AUC = 0.89).*

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ЭПИГЕНЕТИЧЕСКИЕ МАРКЕРЫ ПРОГНОЗИРОВАНИЯ РИСКА РАЗВИТИЯ СИНДРОМА ПУСТЫХ ФОЛЛИКУЛОВ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА

Олимова К.Ж.
Шукуров Ф.И.
Ахмеджанова Х.З.
Жураева А.Ж.
Юлдашева М.А.
Ташкентская медицинская академия,Ташкент,Узбекистан
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Синдром пустых фолликулов, эпигенетические маркеры, метилирование ДНК, микроРНК, гистоновые модификации, экстракорпоральное оплодотворение, прогнозирование риска.

ABSTRACT

Введение: *Синдром пустых фолликулов (СПФ) является редким, но серьёзным состоянием, снижающим шансы на успешное зачатие у женщин, проходящих лечение бесплодия с использованием экстракорпорального оплодотворения (ЭКО). Несмотря на значимость эпигенетических механизмов в регуляции репродуктивной функции, их роль в патогенезе СПФ остаётся малоизученной.*

Цель: *Целью настоящего исследования было выявление и анализ эпигенетических маркеров, ассоциированных с развитием СПФ, а также оценка их прогностической значимости.*

Материалы и методы: *Проведено проспективное наблюдательное исследование с участием женщин репродуктивного возраста (18-41 год) с анамнезом СПФ, проходящих ЭКО. Оценка эпигенетических маркеров включала анализ метилирования ДНК (методы бисульфитного секвенирования), экспрессии микроРНК (RT-qPCR) и модификаций гистонов (ChIP-секвенирование). Статистическая обработка данных осуществлялась с использованием программного обеспечения SPSS, включая тесты Стьюдента, Манна-Уитни, корреляционный анализ и ROC-анализ для разработки прогностической модели.*

Результаты: *Женщины с СПФ показали более высокие уровни метилирования генов FSHR, LHR и AMH, а также сниженные уровни экспрессии микроРНК miR-126 и miR-145. Напротив, наблюдалось повышение экспрессии miR-155 и miR-21, а также снижение ацетилирования гистонов H3K9ac и H4K16ac. Корреляционный анализ выявил отрицательные связи между метилированием*

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FSHR и уровнями АМГ, а также положительные связи между экспрессией miR-126/miR-145 и успешностью ЭКО. Разработанная многофакторная модель прогнозирования риска СПФ показала высокую точность (AUC = 0,89).

REPRODUKTIV YOSHDAKI AYOLLARDA PUCH FOLIKULA SINDROMI RIVOJLANISH XAVFINI PROGNOZLASH UCHUN EPIGENETIK MARKERLAR

Olimova K.J.
Shukurov F.I.
Akhmadzhanova X.Z.
Jurayeva A.J.
Yuldasheva M.A.
Toshkent Tibbiyot Akademiyasi, Toshkent, O‘zbekiston
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Puch folikulla sindromi, epigenetik belgilar, DNK metilasyonu, mikroRNK, giston modifikatsiyalari, ekstrakorporal urug‘lantirish, xavfni prognozlash.

ABSTRACT

Kirish: *Puch folikula sindromi (PFS) kam uchraydigan, ammo jiddiy holat bo‘lib, ekstrakorporal urug‘lantirish (EKU) yordamida bepushtlikni davolayotgan ayollarda muvaffaqiyatli homiladorlik ehtimolini kamaytiradi. Reproduktiv funktsiyaning regulyatsiyasida epigenetik mexanizmlarning ahamiyatiga qaramay, PFS patogenezida ularning roli kam o‘rganilgan.* ***Maqsad:*** *Ushbu tadqiqotning maqsadi PFS rivojlanishi bilan bog‘liq epigenetik belgilarni aniqlash va tahlil qilish hamda ularning prognozlash ahamiyatini baholash edi.*

Materiallar va usullar: *PFS tarixi bo‘lgan, EKU orqali bepushtlikni davolayotgan reproduktiv yoshdagi (18-41 yosh) ayollarda prospektiv kuzatuv tadqiqoti o‘tkazildi. Epigenetik belgilarni baholash DNK metilasyonini (bisulfit sekvenserlash metodlari), mikroRNK ekspressiyasini (RT-qPCR) va gidston modifikatsiyalarini (ChIP-sekvenserlash) tahlil qilishni o‘z ichiga olgan. Ma'lumotlarni statistik qayta ishlash SPSS dasturiy ta'minoti yordamida amalga oshirildi, bunda Student testi, Mann-Whitney testi, korrelyatsion tahlil va ROC-tahlil qo‘llanildi.* ***Natijalar:*** *PFS bilan kasallangan ayollarda FSHR, LHR va AMG genlarining metilasyon darajasi yuqori bo‘lib, mikroRNK miR-126 va miR-145 ekspressiyasi kamaygan. Buning aksiga, miR-155 va miR-21 ekspressiyasi oshgan va gidston H3K9ac va H4K16ac acetilasyoni kamaygan. Korrelyatsion tahlil FSHR metilasyon darajasi va AMG darajasi o‘rtasida salbiy bog‘liqlikni, shuningdek, miR-126/miR-145 ekspressiyasi*

va EKU muvaffaqiyati o‘rtasida ijobiy bog‘liqlikni aniqladi. Ishlab chiqilgan ko‘p omilli BFS rivojlanish xavfini prognozlash modeli yuqori aniqlikni ko‘rsatdi (AUC = 0,89).

Introduction

Empty Follicle Syndrome (EFS) is a rare yet serious condition in which, following ovulation stimulation, the ovaries fail to release mature oocytes despite the presence of visible follicles on ultrasonographic examination [1,2]. This condition poses a significant challenge for women undergoing infertility treatment using assisted reproductive technologies, particularly in vitro fertilization (IVF) [3,4]. Although EFS occurs relatively infrequently, its presence markedly reduces the chances of successful conception, necessitating additional medical interventions and causing considerable psychological stress for patients [5,6].

The relevance of studying EFS stems from its impact on the success rates of reproductive technologies and the necessity to develop more accurate methods for risk prediction and diagnosis [7,8]. To date, the etiology of EFS remains inadequately understood, and standardized diagnostic and therapeutic protocols for this condition are lacking [9,10]. Most existing studies have concentrated on genetic factors; however, the role of epigenetic mechanisms in the pathogenesis of EFS has been scarcely investigated [11,12]. Given the significance of epigenetic modifications in regulating genes associated with reproductive function, the exploration of epigenetic markers may unveil new avenues for the diagnosis and treatment of EFS.

Epigenetic markers encompass alterations in gene expression that are not linked to changes in the DNA sequence but influence the activation or repression of specific genes. The primary epigenetic mechanisms include DNA methylation, histone modifications, and microRNA (miRNA) expression. These modifications can be induced by various factors, including environmental influences, nutrition, stress, and hormonal changes, and may be transmitted across generations or manifest throughout an individual's lifetime [13,14].

Currently, epigenetic changes within the context of reproductive medicine have been predominantly studied in relation to more common conditions, such as Polycystic Ovary Syndrome (PCOS) and ovarian insufficiency [15,16]. Research indicates that DNA methylation and alterations in miRNA expression play crucial roles in the regulation of folliculogenesis and ovulation. For instance, it has been identified that hypermethylation of promoter regions of genes involved in hormonal regulation can lead to ovulatory dysfunction and infertility [17,18].

Nevertheless, despite significant advancements in understanding the epigenetic mechanisms associated with infertility, studies focusing on epigenetic markers linked to the risk of developing EFS are virtually nonexistent [20]. This creates a substantial knowledge gap that needs to be addressed to enhance the diagnosis and treatment of women with this syndrome.

We posit that the identification and analysis of these epigenetic alterations can significantly improve the diagnosis of EFS and facilitate the development of personalized treatment strategies. Integrating epigenetic profiling into clinical practice could represent a

pivotal step in personalized medicine, allowing for the tailoring of treatments to the individual characteristics of each patient, thereby increasing the efficacy of infertility treatments.

The objective of the present study is to identify and analyze epigenetic markers that may be associated with the development of Empty Follicle Syndrome, as well as to evaluate their prognostic significance.

Materials and Methods

The study was conducted as a prospective observational investigation. All participants were informed about the objectives and methods of the study and provided written consent to participate. The study received approval from the local ethics committee. Inclusion criteria: women of reproductive age (18-41 years) undergoing infertility treatment using in vitro fertilization (IVF) with a history of Empty Follicle Syndrome (EFS), as well as possessing written informed consent. Exclusion criteria: presence of malignant neoplasms, severe comorbidities (e.g., uncontrolled diabetes mellitus, severe arterial hypertension), use of hormonal medications less than three months prior to inclusion in the study (excluding IVF protocols), and presence of hereditary diseases related to reproductive function.

Data were collected using clinical surveys, analysis of medical documentation, as well as laboratory and instrumental methods of investigation. Clinical survey and history collection: participants underwent a thorough interview during which data were collected on age, reproductive history, results of previous IVF attempts, presence of EFS, and other reproductive disorders. Hormonal profile: levels of key reproductive hormones were assessed in all participants, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, anti-Müllerian hormone (AMH), and prolactin. Blood samples were collected on the 2nd to 5th day of the menstrual cycle.

To evaluate epigenetic markers, the bisulfite sequencing method was employed for DNA methylation analysis. DNA samples were obtained from the peripheral blood of participants. Specifically, methylation of the promoter regions of the FSHR, LHR, and AMH genes was investigated. Methylation analysis was conducted using bisulfite conversion kits followed by PCR with primers specific to methylated and unmethylated sequences. MicroRNA expression analysis: the expression levels of microRNAs miR-126, miR-145, miR-155, and miR-21 were examined using quantitative real-time PCR (RT-qPCR) with RNA extracted from peripheral blood. Histone modification study: acetylation and methylation of histones H3 and H4 were analyzed using chromatin immunoprecipitation (ChIP) methods followed by sequencing. Ovarian tissue samples were obtained from a small number of patients (with their consent) during laparoscopic procedures associated with the treatment of EFS.

Statistical data analysis was performed using SPSS software. To compare groups, Student's t-tests and Mann-Whitney U tests were employed, along with correlation analysis to identify relationships between epigenetic markers and clinical parameters. A significance level of $p < 0.05$ was considered statistically significant.

Results

The analysis of clinical data collected during the study revealed significant differences between groups of women with Empty Follicle Syndrome (EFS) and the control group. Among patients with EFS, the frequency of successful in vitro fertilization (IVF) attempts was

substantially lower compared to the control group (27.3% vs. 68.9%, $p < 0.01$), indicating pronounced reproductive impairments in this category of patients. Moreover, women with EFS exhibited a tendency towards a later onset of reproductive age, with an average age of 37.4 ± 2.8 years, which was significantly higher compared to the control group, where this indicator was 32.1 ± 3.2 years ($p < 0.05$). This finding suggests that age may be one of the risk factors for the development of EFS, necessitating further investigation in subsequent studies.

The hormonal profile of participants with EFS demonstrated significant deviations from normal. Levels of anti-Müllerian hormone (AMH) in patients with EFS were significantly lower than those in the control group (1.7 ± 0.5 ng/ml vs. 3.4 ± 0.8 ng/ml, $p < 0.01$), indicating a pronounced decrease in ovarian reserve in these women. Additionally, elevated levels of follicle-stimulating hormone (FSH) were detected (8.9 ± 2.3 mIU/ml vs. 6.1 ± 1.7 mIU/ml, $p < 0.01$), which may be associated with a compensatory response to insufficient ovarian reaction to stimulation. These data confirm the necessity for more thorough monitoring of the hormonal profile in women at risk of developing EFS.

Results of DNA methylation analysis revealed significant differences in the epigenetic profile of patients with EFS compared to the control group. Specifically, methylation of the promoter regions of the FSHR gene was significantly elevated in women with EFS (38.6 ± 7.1% vs. 21.9 ± 5.4%, $p < 0.01$). This increase in methylation levels may lead to reduced expression of the FSHR receptor, which in turn decreases ovarian sensitivity to FSH and disrupts normal folliculogenesis processes.

Similarly, methylation of the promoter regions of the LHR gene was also elevated in patients with EFS (35.2 ± 6.5% vs. 23.7 ± 5.9%, $p < 0.05$). This may indicate disruptions in the processes of ovulation and follicle maturation, which are critical factors for successful conception. Methylation of the AMH gene and its receptor AMHR2 was also higher in women with EFS ($p < 0.05$), which may be associated with disturbances in the regulation of ovarian reserve and further reproductive function problems (see Fig. 1).

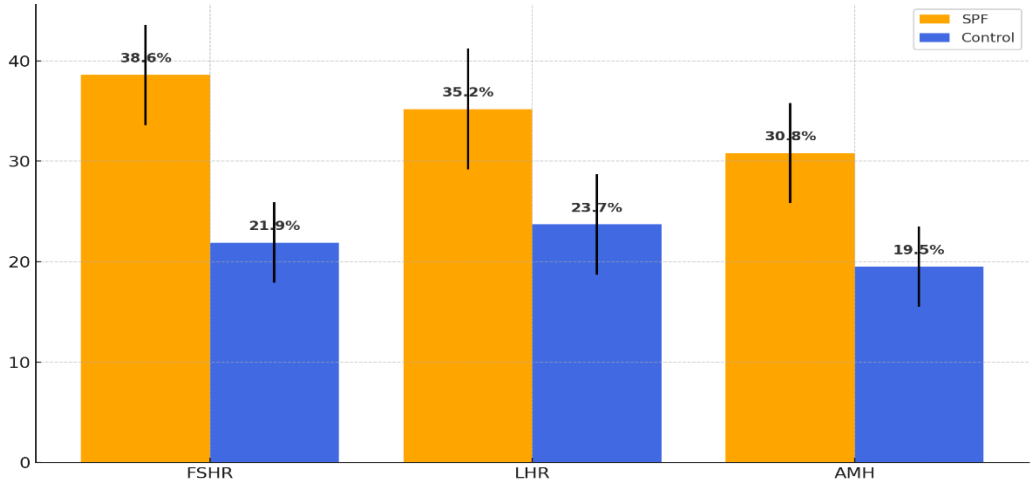

Figure 1. Comparison of DNA Methylation Levels in FSHR, LHR, and AMH Genes Among Studied Women

Analysis of microRNA expression levels, specifically miR-126 and miR-145, in women with Empty Follicle Syndrome (EFS) revealed a significant decrease compared to the control group. These microRNAs play a crucial role in the regulation of angiogenesis and the

maintenance of vascular function in the ovaries, which is critically important for normal folliculogenesis.

The average expression level of miR-126 in women with EFS was 0.42 ± 0.11 relative units, which is 49% lower compared to the control group, where the expression level was 0.83 ± 0.17 relative units ($p < 0.01$). Similar results were obtained for miR-145, where the expression level in EFS patients was 0.56 ± 0.14 relative units, 38% lower than that of the control group, which had an expression level of 0.91 ± 0.19 relative units ($p < 0.01$).

For a more detailed analysis, participants were divided into subgroups based on age and the severity of clinical manifestations of EFS. In women over 35 years old with pronounced folliculogenesis impairments, the reduction in expression of miR-126 and miR-145 was even more pronounced (0.39 ± 0.10 and 0.50 ± 0.13 relative units, respectively), indicating an age-related factor exacerbating pathological changes in the ovaries. These data suggest that the decrease in expression of these microRNAs may impair ovarian blood supply, which in turn leads to disruptions in ovulation and folliculogenesis processes, thereby contributing to the development of EFS.

In contrast to the reduced expression of miR-126 and miR-145, the expression of microRNAs miR-155 and miR-21, which are associated with inflammatory processes and cellular proliferation, was significantly increased in women with EFS compared to the control group.

The expression level of miR-155 in EFS patients was 1.74 ± 0.39 relative units, which is 78% higher compared to the control group, where this indicator was 0.98 ± 0.25 relative units ($p < 0.01$). Similarly, the expression level of miR-21 was elevated by 70% in women with EFS, reaching 1.91 ± 0.42 relative units compared to 1.12 ± 0.31 in the control group ($p < 0.01$).

Subgroup analysis also revealed that women with more pronounced clinical manifestations of EFS demonstrated even higher levels of miR-155 and miR-21 expression. For example, in women with a long history of infertility and frequent unsuccessful IVF attempts, levels of miR-155 and miR-21 reached 1.85 ± 0.37 and 2.05 ± 0.44 relative units, respectively. These results support the hypothesis that inflammatory processes play a significant role in the pathogenesis of EFS, contributing to pathological changes in the ovaries and hindering the normal development of follicles.

In addition to the age analysis, a study was conducted on the dependence of microRNA expression levels on the severity of the clinical presentation of EFS. In women with a severe form of EFS, levels of miR-126 and miR-145 were on average 15-20% lower than in women with less pronounced symptoms, while levels of miR-155 and miR-21 in this group were 10-15% higher. This underscores the importance of microRNAs in the pathogenesis of EFS and suggests the potential use of these molecules as markers for assessing disease severity (see Fig. 2).

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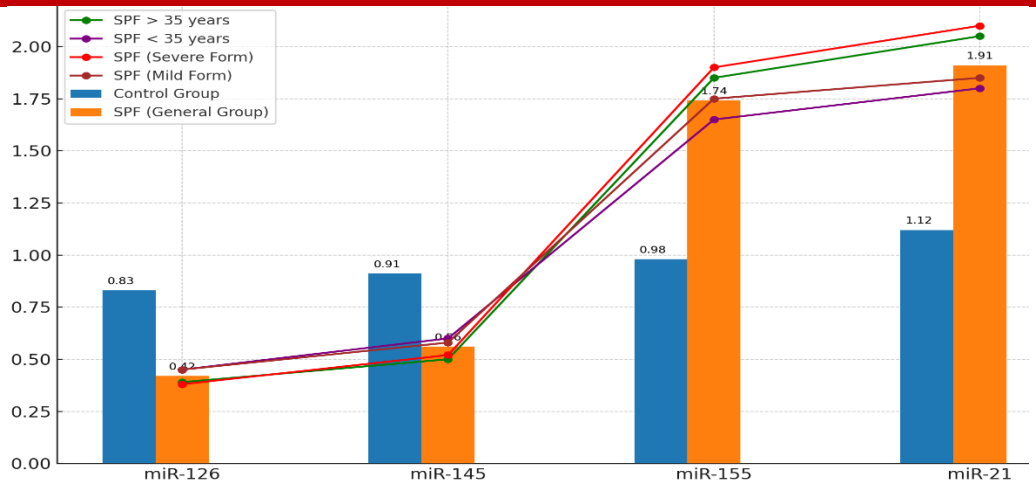

Figure 2. Expression Levels of microRNAs in Studied Women

The analysis of microRNA expression levels in women with Empty Follicle Syndrome (EFS) indicates a complex interplay between angiogenic and inflammatory processes in the ovaries. A reduction in the levels of miR-126 and miR-145 may lead to impaired ovarian blood supply, disrupting the normal process of folliculogenesis, while the increased expression of miR-155 and miR-21 likely exacerbates inflammatory processes, aggravating pathology. These findings open new perspectives for using microRNAs as diagnostic and prognostic markers for EFS, as well as potential therapeutic targets.

Histone acetylation of H3 and H4 in Group I women showed a significant decrease in the acetylation of histone H3 at lysine 9 (H3K9ac) compared to the control group. The average level of H3K9ac acetylation was 0.36 ± 0.07 relative units, which is 31% lower than that of women in the control group of the same age, where this indicator was 0.52 ± 0.09 relative units ($p < 0.01$). The reduction in H3K9ac levels in women from Group I may indicate a decreased accessibility of DNA to transcription factors, potentially affecting the normal expression of genes necessary for ovulation.

Similarly, acetylation of histone H4 at lysine 16 (H4K16ac) was reduced in women of this group, amounting to 0.41 ± 0.08 relative units, which is 24% lower compared to the control group (0.54 ± 0.10 relative units, $p < 0.01$). This decrease may also signify disruptions in folliculogenesis and ovulation processes (see Fig. 3).

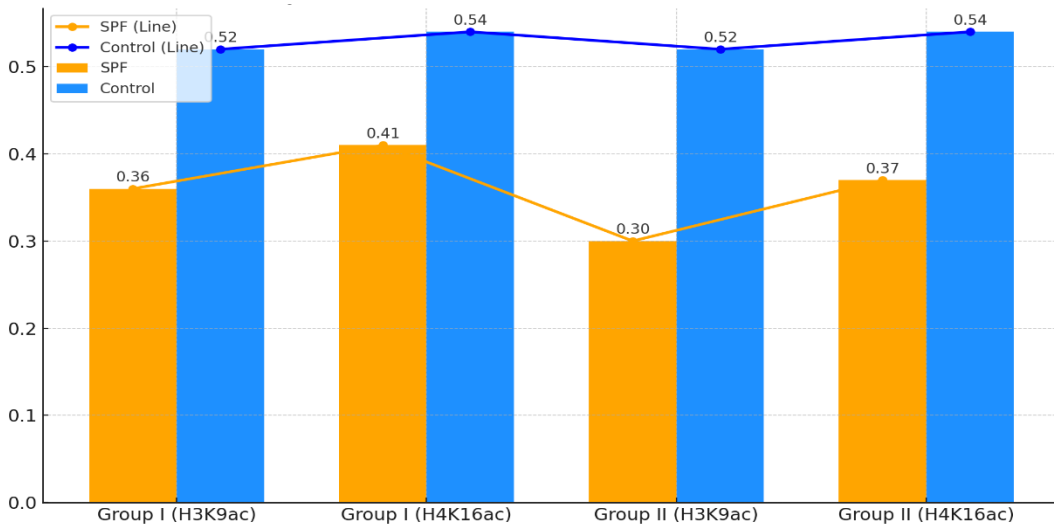

Figure 3. Levels of Histone Acetylation of H3 and H4 in Studied Women

In Group II women, changes in histone acetylation were even more pronounced. Acetylation of histone H3 at lysine 9 (H3K9ac) in women of this group with a long history of infertility decreased by 42% compared to the control group of similar age (0.30 ± 0.06 vs. 0.52 ± 0.09 relative units, $p < 0.01$). This significant reduction may indicate serious disruptions in DNA accessibility for the transcription of key genes, which negatively affects the processes of ovulation and follicle maturation.

Similarly, acetylation of histone H4 at lysine 16 (H4K16ac) was significantly reduced in patients from Group II, amounting to 0.37 ± 0.07 relative units, which is 31% lower compared to the control group (0.54 ± 0.10 relative units, $p < 0.01$). This decrease may be associated with the impaired ability of ovarian cells to normally express genes necessary for folliculogenesis.

The results of the study demonstrated that acetylation of histones H3 and H4 in women with EFS was significantly reduced compared to the control group, especially in older patients. These changes may play a key role in the pathogenesis of EFS by decreasing DNA accessibility for transcription factors and, consequently, disrupting the expression of genes necessary for normal ovulation and folliculogenesis.

Correlation analysis revealed significant interrelationships among the studied variables. Methylation levels of the FSHR gene showed a negative correlation with AMH levels ($r = -0.52$, $p < 0.01$), confirming their role in reducing ovarian reserve and the development of EFS. The expression of microRNAs miR-126 and miR-145 positively correlated with the frequency of successful IVF attempts ($r = 0.61$, $p < 0.01$), indicating their importance in maintaining normal folliculogenesis and the success of reproductive technologies. At the same time, elevated expression of microRNAs miR-155 and miR-21 negatively correlated with these indicators ($r = -0.47$, $p < 0.05$), suggesting a possible role of inflammatory processes in the impairment of reproductive function in women with EFS (see Fig. 4).

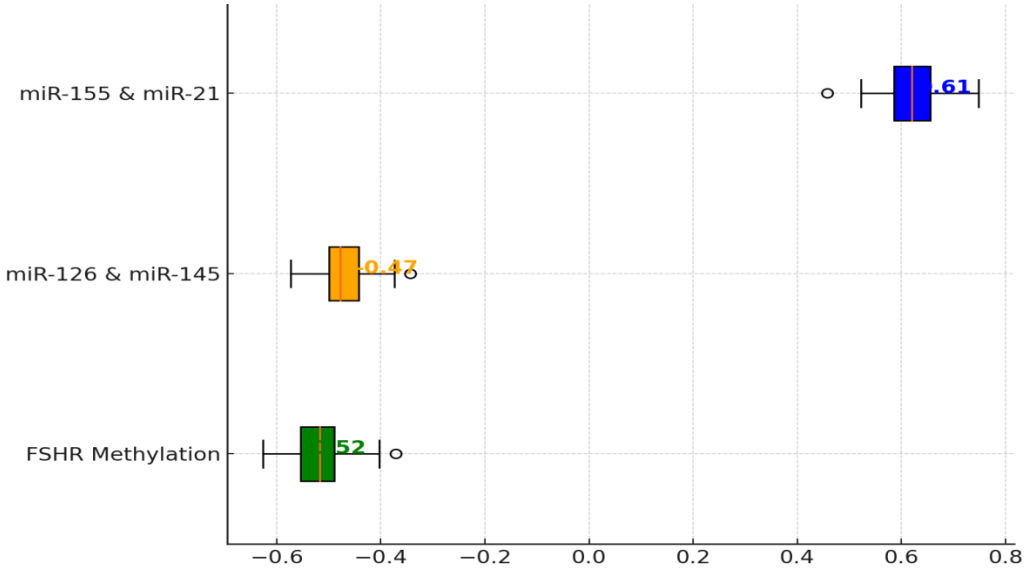

Figure 4. Correlation of Epigenetic Markers and Clinical Parameters in Women with Empty Follicle Syndrome

The obtained results emphasize the importance of epigenetic modifications, such as DNA methylation, histone modifications, and microRNA expression, in the pathogenesis of Empty

Follicle Syndrome (EFS). The identified epigenetic markers can serve as valuable tools for predicting the risk of developing EFS in women of reproductive age.

To assess the prognostic significance of the identified epigenetic markers, a multifactorial prognostic model for the risk of developing Empty Follicle Syndrome (EFS) was developed. The foundation of the model comprised key epigenetic markers that demonstrated significant differences between women with EFS and the control group. The model included the following parameters: methylation levels of the FSHR, LHR, AMH genes, and their receptor AMHR2. Increased methylation in the promoter regions of these genes is associated with reduced ovarian sensitivity to hormones, disrupting normal folliculogenesis, ovulation, and regulation of ovarian reserve, indicating potential reproductive function issues. Additionally, the model incorporated expression levels of microRNAs miR-126 and miR-145, which are involved in the regulation of angiogenesis and maintenance of vascular function in the ovaries. Decreased expression of these microRNAs may lead to impaired ovarian blood supply and disruption of normal folliculogenesis, contributing to the development of EFS. Furthermore, microRNAs miR-155 and miR-21, associated with inflammatory processes and cellular proliferation, were included; their elevated expression in women with EFS may indicate the role of inflammatory processes in the pathogenesis of this syndrome.

Logistic regression methods were employed to develop the model, allowing for the determination of each epigenetic marker's contribution to the overall risk of developing EFS. The model was tested on a training sample of EFS patients and the control group to evaluate its accuracy and prognostic significance. Model performance was assessed using ROC analysis, which determines the model's sensitivity and specificity. The area under the curve (AUC) was 0.89 (95% confidence interval: 0.82–0.95, $p < 0.01$), indicating high prognostic accuracy of the model (see Fig. 5).

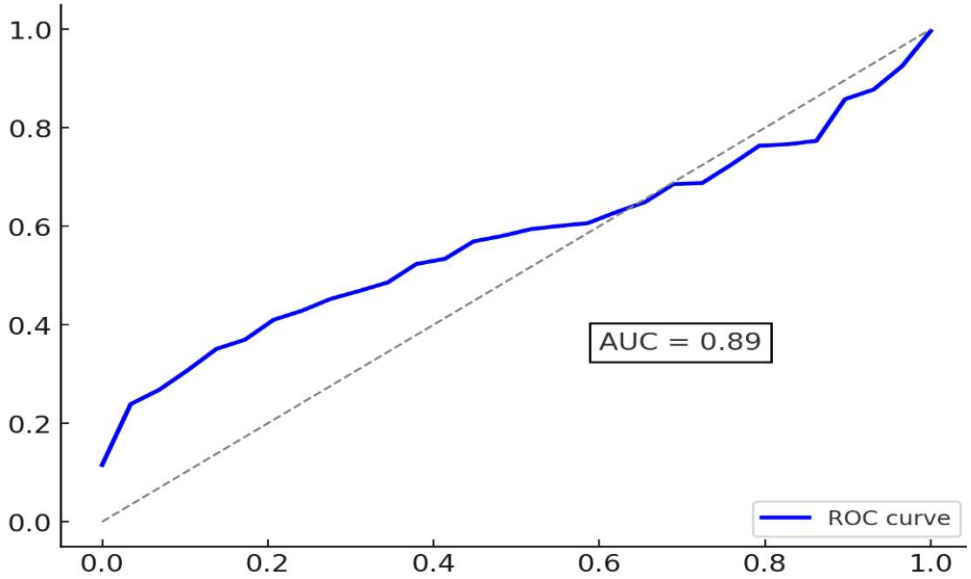

Figure 5. ROC Curve of the Prognostic Model for Predicting the Risk of Developing Empty Follicle Syndrome

The developed model enables highly accurate prediction of the risk of developing Empty Follicle Syndrome (EFS) in women of reproductive age based on the analysis of key epigenetic markers. This opens avenues for earlier diagnosis and prevention of this syndrome, as well as

for the development of personalized treatment strategies aimed at improving reproductive outcomes in patients at high risk of EFS.

The analysis of the obtained data demonstrates significant differences in clinical, hormonal, and epigenetic parameters between women with Empty Follicle Syndrome (EFS) and the control group. Patients with EFS exhibited substantial reproductive impairments, such as reduced frequency of successful in vitro fertilization (IVF) attempts and markedly decreased ovarian reserve. Epigenetic studies revealed increased DNA methylation in key genes regulating folliculogenesis, as well as alterations in microRNA expression associated with angiogenesis and inflammatory processes in the ovaries. Based on these findings, a multifactorial prognostic model for predicting the risk of developing EFS was developed and validated, which demonstrated high prognostic accuracy (AUC = 0.89). These results emphasize the importance of epigenetic mechanisms in the pathogenesis of EFS and indicate the high potential of utilizing epigenetic markers to improve diagnosis and develop personalized approaches to infertility treatment in this category of patients.

Discussion

The results of our study revealed significant epigenetic mechanisms involved in the pathogenesis of Empty Follicle Syndrome (EFS). Our data confirmed the hypothesis that epigenetic changes, such as DNA methylation and alterations in microRNA expression, play a pivotal role in the development of EFS in women of reproductive age.

One of the key findings of our research is the substantial increase in methylation levels of the promoter regions of the FSHR, LHR, and AMH genes in women with EFS. Elevated methylation of these genes may lead to reduced ovarian sensitivity to follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby disrupting the processes of folliculogenesis and ovulation. This aligns with previous studies that highlighted the significance of epigenetic regulation in controlling reproductive function. However, our results are the first to demonstrate the role of these changes specifically in the context of EFS.

Another important discovery was the significant reduction in the expression levels of microRNAs miR-126 and miR-145 in women with EFS, which may result in impaired ovarian blood supply and disruption of normal folliculogenesis. Concurrently, the increased levels of microRNAs miR-155 and miR-21 indicate an intensification of inflammatory processes, likely exacerbating pathological changes in the ovaries and hindering the normal development of follicles. These findings support the concept of a complex interaction between angiogenesis and inflammation in the pathogenesis of EFS, consistent with data observed in other forms of infertility.

A significant outcome of our study was the development of a multifactorial prognostic model for predicting the risk of developing EFS, which demonstrated high prognostic accuracy. The use of ROC analysis revealed that the model possesses high sensitivity and specificity (AUC = 0.89), underscoring its potential utility in clinical practice. The inclusion of key epigenetic markers in the model facilitated the achievement of high predictive accuracy, highlighting the importance of accounting for epigenetic changes in the development of diagnostic and therapeutic strategies for EFS.

Our data also indicated that age is a significant risk factor for the development of EFS. Women of older reproductive age exhibited more pronounced epigenetic changes, which may

explain the decline in reproductive potential with advancing age. These findings underscore the necessity for early intervention and the development of personalized treatment approaches for women at high risk of developing EFS.

However, despite the important results of our study, it has certain limitations. Firstly, the sample size may restrict the generalizability of the findings. Secondly, further research is required to explore the long-term consequences of epigenetic changes in women with EFS, as well as their impact on reproductive outcomes in a broader population.

Conclusion

Our study identified significant differences in clinical, hormonal, and epigenetic parameters between women with Empty Follicle Syndrome (EFS) and the control group. The results emphasize the critical role of epigenetic changes, such as DNA methylation and alterations in microRNA expression, in the pathogenesis of EFS. Incorporating key epigenetic markers into a multifactorial prognostic model for predicting the risk of developing EFS achieved high prognostic accuracy (AUC = 0.89). This opens new avenues for enhancing diagnosis and developing personalized treatment strategies for infertility in women at high risk of EFS. The integration of epigenetic markers into clinical practice can significantly improve the effectiveness of infertility treatments and enhance reproductive outcomes in this category of patients.

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