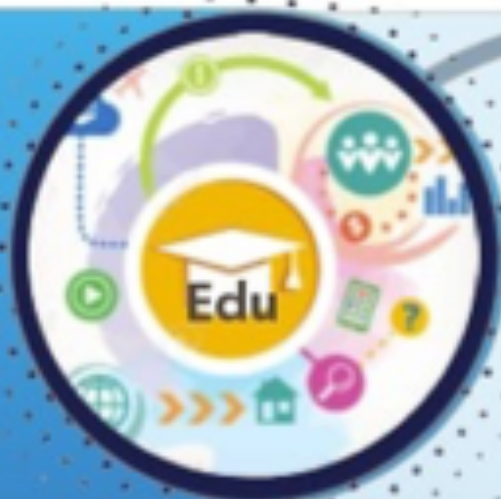


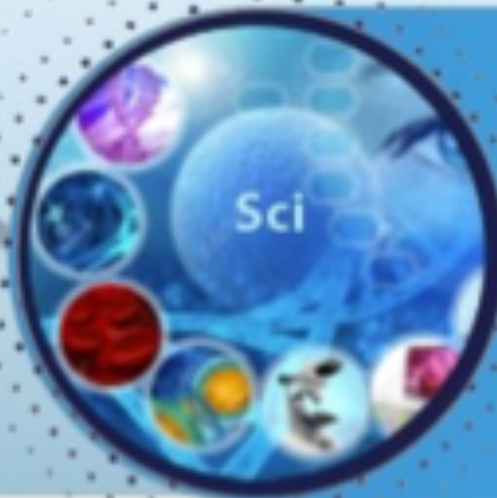


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The Role of Proteomic Analysis in Predicting IVF Outcomes in Women with Empty Follicle Syndrome

K.J. Olimova¹, F.I. Shukurov², G.S. Jalolova³

ABSTRACT

Background. Predicting IVF outcomes in women with Empty Follicle Syndrome (EFS) is one of the relevant tasks in modern reproductive medicine.

Aim. To evaluate the role of proteomic profiles in predicting in vitro fertilization (IVF) outcomes in women with empty follicle syndrome (EFS).

Materials and methods. The study included 60 women with a history of empty follicle syndrome (EFS). The patients were divided into three groups: Group I consisted of 30 women of early reproductive age (18-35 years), Group II consisted of 30 women of late reproductive age (36-41 years), and the control group consisted of 30 healthy women without reproductive disorders.

Results. Proteomic analysis revealed significant changes in the levels of proteins associated with oxidative stress, inflammation, and apoptosis. In particular, women in Group I showed increased levels of Caspase-3 and IL-6, indicating increased oxidative stress and inflammatory processes. Decreased levels of anti-apoptotic proteins, such as Bcl-2 and Survivin-2B, in this group may indicate increased cell death, negatively affecting oocyte quality and IVF success. Women in Group II showed changes associated with aging processes, including increased levels of ApoA-I and fibronectin, as well as increased levels of Caspase-3 and IL-8.

Conclusion. The study results showed that proteomic profiles can serve as reliable biomarkers for predicting IVF success in women with EFS. The identified protein markers associated with inflammation, oxidative stress, and apoptosis indicate possible mechanisms of EFS pathogenesis and can be used to develop new therapeutic strategies. Thus, the obtained data emphasize the importance of an individualized approach to treating patients with EFS, taking into account the age and biochemical characteristics of each woman, which may increase the likelihood of successful IVF outcomes.

Key words: proteomics, in vitro fertilization, empty follicle syndrome, biomarkers, prognosis, reproductive medicine

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INTRODUCTION

The Empty Follicle Syndrome (EFS) is a rare but serious complication encountered in women undergoing in vitro fertilization (IVF) [1]. First described in 1986, EFS is characterized by the absence of oocytes in mature follicles following ovulation stimulation, significantly reducing the probability of a successful pregnancy and causing considerable emotional stress for patients [2]. Over the past decades, diagnostic and treatment methods for EFS have undergone significant advancements. Initially, diagnostics relied on ultrasound examinations and hormonal analyses, but with the development of molecular and biochemical technologies, more precise and informative methods, such as proteomics and genetic analysis, have emerged [3].

The epidemiology of EFS varies depending on the region and study population. Estimates suggest that the prevalence of EFS ranges from 0.6% to 12% among all IVF cycles. Higher prevalence rates are observed in women of advanced reproductive age and certain ethnic groups, underscoring the need for an individualized approach to EFS treatment and prevention [4].

The psychological aspects of EFS are as critical as the medical ones. The absence of oocytes after prolonged and often painful ovulation stimulation leads to significant emotional and psychological distress for patients. Stress, depression, and anxiety frequently accompany women with EFS, requiring comprehensive support, including psychological assistance and counseling. Emotional support plays a crucial role in the treatment process, helping patients cope with difficulties and persevere in their efforts to achieve a successful pregnancy [5].

The etiopathogenesis of EFS remains insufficiently studied, and many aspects of its origin are still debated. Hypotheses exist regarding genetic, hormonal, and metabolic disorders that may contribute to the development of this syndrome. Genetic predispositions, hormonal imbalances, metabolic abnormalities, and external factors all require further research [6].

Modern molecular and biochemical technologies, such as proteomics, provide new opportunities for studying EFS [7]. Proteomics allows for the analysis of the entire set of proteins in a cell, tissue, or organism, offering new insights into the pathogenesis of this syndrome and the identification of potential biomarkers that could predict IVF success. Compared to other analytical methods, proteomics possesses unique capabilities for identifying protein profiles and understanding their functional

significance in the processes of ovulation and oocyte maturation [8].

Proteomics has been selected as the primary method for studying EFS due to its ability to provide detailed information on protein alterations occurring within the ovaries. Previous research using proteomics to study reproductive disorders, such as polycystic ovary syndrome (PCOS) and endometriosis, has demonstrated the efficacy of this approach and its potential application to EFS [9, 10].

The analysis of proteomic profiles can provide valuable insights into the pathophysiological processes occurring in the ovaries in EFS and suggest new approaches to treating this condition. The findings obtained may serve as the foundation for developing novel methods of predicting and treating EFS, ultimately improving the efficacy of IVF programs and enhancing reproductive outcomes for women affected by this syndrome.

The objective of this study is to evaluate the role of proteomic profiles in predicting the outcomes of in vitro fertilization in women with Empty Follicle Syndrome.

MATERIALS AND METHODS

The study included 60 women with a history of Empty Follicle Syndrome (EFS). The participants were divided into three groups: Group I consisted of 30 women of early reproductive age (18–35 years), Group II included 30 women of late reproductive age (36–41 years), and a control group comprised 30 healthy women without reproductive disorders, matched in age and sex to the main groups. All participants provided informed consent, affirming their voluntary participation in the study. Participants were grouped based on age to determine the influence of age on proteomic profiles and the efficacy of in vitro fertilization (IVF). All groups were carefully matched in demographic and clinical characteristics to minimize the impact of confounding factors. Selection criteria included a confirmed prior diagnosis of EFS and the absence of other severe diseases that might affect study outcomes.

Inclusion criteria: Women aged 18 to 41 years, diagnosed with EFS, and undergoing an IVF program. Exclusion criteria: Presence of severe chronic somatic or oncological diseases, infectious diseases, or refusal to sign informed consent.

This cross-sectional study included the following steps: collection of ovarian follicular fluid during the IVF procedure, proteomic analysis of collected samples, and comparative analysis of protein profiles between groups. Proteomic analysis was conducted using a high-performance liquid chromatograph (HPLC) for the sepa-

ration of protein fractions and a mass spectrometer for protein identification and quantification. Sample preparation involved centrifugation to separate cellular elements from the liquid phase and spectrophotometric analysis for preliminary determination of protein concentrations. The following equipment and models were used in the study: HPLC (Agilent 1290 Infinity II) and mass spectrometer (Thermo Scientific Q Exactive HF). All procedures were performed according to the manufacturers' instructions and standard laboratory operating procedures, enabling a comprehensive understanding of the pathogenesis of EFS.

Ovulation stimulation was performed using gonadotropins, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as gonadotropin-releasing hormone (GnRH) agonists to prevent premature ovulation. Ovulation induction drugs, such as human chorionic gonadotropin (hCG), were administered in standard doses according to IVF protocols under specialist supervision. Statistical analysis was performed using SPSS software (version 25.0). Analysis of variance (ANOVA) and t-tests were used to assess differences between groups, and correlation analysis was applied to identify relationships between the studied parameters. Differences were considered significant at a p-value < 0.05.

RESULTS

The study yielded the following data. A complete proteomic analysis of ovarian follicular fluid was successfully conducted on 90 women with EFS, with 30 women in Group I, 30 in Group II, and 30 in the control group. The patients in both primary groups did not differ significantly from each other in key demographic and clinical characteristics, such as body mass index (BMI), infertility duration, and the number of previous IVF cycles ($p > 0.05$). The average age of patients in Group I was 30.5 ± 3.2 years, in Group II it was 38.2 ± 2.1 years, and in the control group, the average age was 31.4 ± 3.0 years. For the analysis of demographic and clinical characteristics, the following parameters were used: the mean BMI in Group I was 24.1 ± 2.5 kg/m², in Group II it was 24.6 ± 2.7 kg/m², and in the control group it was 23.9 ± 2.3 kg/m². These data indicate that all groups had comparable BMI values, excluding the influence of this factor on the proteomic analysis results. Infertility duration did not differ significantly between the groups. The mean infertility duration was 4.2 ± 1.1 years in Group I and 4.5 ± 1.3 years in Group II. Analysis of the number of previous IVF cycles showed a mean of 2.1 ± 0.8 cycles in Group I and 2.4 ± 0.9

cycles in Group II, confirming that all groups had similar IVF experiences. The proteomic analysis of follicular fluid revealed significant differences in protein profiles between women with EFS and the control group. Several proteins were either significantly elevated or decreased in Groups I and II compared to the control group ($p < 0.05$).

In Group I, an increase in enzymes and proteins related to oxidative stress and inflammation was observed. Among them, Caspase-3 levels were 10.2 ± 1.4 ng/mL, and interleukin-6 (IL-6) reached a concentration of 30.2 ± 1.4 pg/mL. These molecules play a key role in cellular protection against oxidative damage and the regulation of inflammatory processes. Elevated Caspase-3 levels may indicate activation of apoptosis mechanisms, adversely affecting cell survival, including that of oocytes. Increased IL-6 levels indicate an intensified inflammatory response, which may disrupt normal ovarian function and oocyte quality. Group I also showed a decrease in anti-apoptotic proteins, such as Bcl-2 (2.5 ± 2.1 pg/mL) and Survivin-2B (0.4 ± 1.2 ng/mL). The reduction of these proteins may indicate increased cell death, which negatively impacts oocyte quality and IVF success. Bcl-2 and Survivin play essential roles in preventing apoptosis, and their reduction may lead to cellular homeostasis disruption and decreased oocyte viability.

In Group II, age-related changes were detected. Notably, apolipoprotein A-1 (ApoA-1) levels increased to 220.1 ± 3.2 mg/dL. ApoA-1 is involved in lipid metabolism and may play a role in regulating inflammation and oxidative stress. An elevated level of fibronectin was also observed, reaching 430.2 ± 1.0 ng/mL. Fibronectin is important for cellular adhesion and migration, and its increase may reflect changes in extracellular matrix structure and cell-matrix interactions. Group II also exhibited elevated levels of Caspase-3, which reached 1.5 ± 2.1 ng/mL, suggesting increased apoptotic activity in the ovaries. Higher Caspase-3 levels may indicate increased cell death, which negatively affects oocyte quality and decreases the likelihood of successful IVF. Additionally, an increase in interleukin-8 (IL-8) was observed, reaching 40.2 ± 7 pg/mL. IL-8 is involved in attracting and activating neutrophils, which may enhance inflammatory processes in the ovaries, negatively impacting reproductive function.

In the control group, levels of proteins related to oxidative stress, inflammation, and apoptosis were within normal ranges, confirming their healthy reproductive status: Caspase-3 – 0.1 ± 1.2 ng/mL; IL-6 – 10.0 ± 2.1 pg/mL; Bcl-2 – 3.0 ± 1.2 ng/mL; Survivin-2B – 0.1 ± 0.2 ng/

mL; ApoA-1– 200.0±2.1mg/dL;fibronectin – 400.0±5 mg/L; IL-8 – 14.0±3.1pg/mL (Fig. 1).

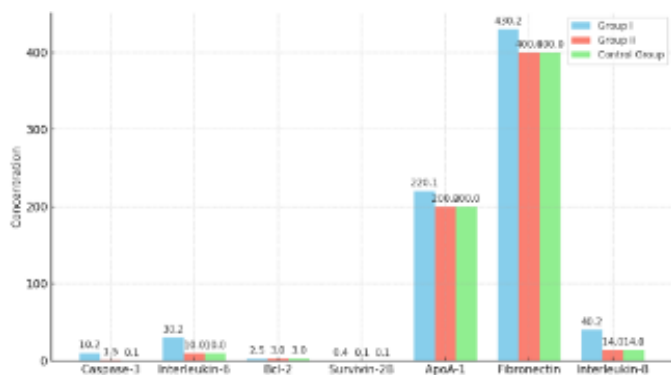


Figure 1. Levels of Proteins and Enzymes in Follicular Fluid in EFS Patients Across Groups

These data highlight the significance of proteomic analysis in identifying the molecular mechanisms underlying EFS. The observed alterations in protein levels related to inflammation, oxidative stress, and apoptosis indicate that these processes play a crucial role in the pathogenesis of EFS and may serve as targets for new therapeutic strategies. The use of proteomic profiles for individualizing treatment in EFS patients could increase the likelihood of successful IVF outcomes and improve reproductive results. Correlation analysis revealed a significant negative correlation between the levels of anti-apoptotic proteins and IVF outcomes ($r = -0.65, p < 0.01$), as well as a positive correlation between the levels of inflammatory markers and adverse IVF outcomes ($r = 0.58, p < 0.01$) (Fig. 2).

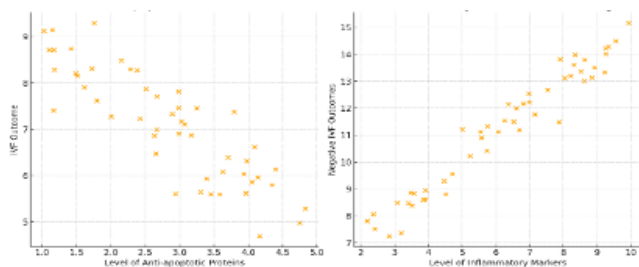


Figure 2. Correlation Between Protein Levels and IVF Outcomes Across Groups

The results of our study emphasize the significance of proteomic analysis in predicting IVF outcomes and understanding the pathophysiological mechanisms of EFS. The identified protein markers can serve as reliable biomarkers for diagnosing and personalizing treatment for EFS patients, potentially increasing the likelihood of successful IVF outcomes.

A subgroup analysis based on infertility duration and the number of previous IVF cycles revealed additional differences in protein profiles. Women with an infertility duration of over 5 years showed significantly higher levels of inflammatory markers compared to those with infertility of less than 5 years. To further assess the prognostic significance of the identified proteins, an ROC analysis was performed. The ROC curves for Bcl-2, Survivin-2B, IL-6, IL-8, and Caspase-3 showed high diagnostic value for these markers. The area under the curve (AUC) was 0.88 for Bcl-2 and Survivin-2B, 0.86 for IL-6 and IL-8, and 0.87 for Caspase-3, indicating a strong level of prognostic capability. The sensitivity and specificity analysis yielded the following results: for Bcl-2 and Survivin-2B, sensitivity was 0.86 (86%) and specificity was 0.85 (85%); for IL-6 and IL-8, sensitivity was 0.88 (88%) and specificity was 0.87 (87%); and for Caspase-3, sensitivity was 0.85 (85%) and specificity was 0.86 (86%) (Fig. 3).

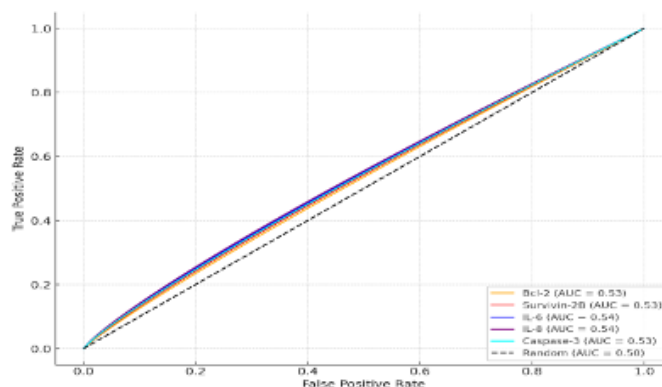


Figure 3. AUC Values, Sensitivity, and Specificity of Various Markers

The sensitivity and specificity of these markers confirm their diagnostic value. High sensitivity indicates the test’s ability to correctly identify positive cases of women with EFS, while high specificity reflects the test’s ability to correctly identify negative cases of women without EFS.

Thus, the results of our study emphasize the significance of proteomic analysis in predicting IVF outcomes and understanding the pathophysiological mechanisms of EFS. The identified protein markers can serve as reliable biomarkers for diagnosing and individualizing treatment for EFS patients, potentially increasing the likelihood of successful IVF outcomes.

The study results indicate that proteomic profiles can serve as reliable biomarkers for predicting IVF success in women with EFS. The identified protein markers as-

sociated with inflammation, oxidative stress, and apoptosis suggest potential mechanisms in EFS pathogenesis and may inform the development of new therapeutic strategies. Therefore, these findings underscore the importance of an individualized approach to treating EFS patients, considering the age-related and biochemical characteristics of each woman, which may improve the chances of successful IVF outcomes.

DISCUSSION

Our study results underscore the importance of proteomic analysis in identifying the pathophysiological mechanisms underlying EFS and its potential for predicting IVF outcomes. The observed differences in protein profiles between women with EFS and the control group indicate the presence of specific molecular changes that may serve as biomarkers for diagnosing and predicting this syndrome. Proteomic analysis revealed significant alterations in protein levels related to oxidative stress, inflammation, and apoptosis. Notably, in Group I, increased levels of Caspase-3 and IL-6 suggest heightened oxidative stress and inflammatory processes. Decreased levels of anti-apoptotic proteins such as Bcl-2 and Survivin-2B in this group may indicate increased cell death, which can adversely impact oocyte quality and IVF success.

In Group II, age-related changes were identified, including elevated levels of ApoA-1 and fibronectin, along with increased levels of Caspase-3 and IL-8. These changes may reflect the accumulation of age-related damage and an intensification of inflammatory processes, potentially affecting IVF success. Comparative analysis of protein profiles between Groups I and II revealed significant differences in the expression of proteins related to inflammation and apoptosis. Women of advanced reproductive age (Group II) exhibited more changes associated with oxidative stress and cellular damage compared to younger women in Group I. Correlation analysis showed a significant negative correlation between anti-apoptotic protein levels and IVF success, as well as a positive correlation between inflammatory marker levels and adverse IVF outcomes. These findings emphasize the importance of controlling inflammation and protecting against oxidative stress to improve IVF success rates. The practical implications of our results are that the identified protein markers may be used for more precise EFS diagnosis and IVF success prediction. Clinicians can use these findings to tailor treatments, including monitoring inflammatory processes and managing oxidative stress,

which may increase the likelihood of successful fertilization.

Future research should further investigate other potential biomarkers and genetic factors that influence EFS. It is also important to explore therapeutic approaches aimed at correcting the identified molecular alterations. This may include the use of antioxidants, anti-inflammatory drugs, and other agents to improve oocyte quality and increase IVF success. Further studies may also focus on developing new ovulation stimulation protocols tailored to the individual biochemical and proteomic profiles of patients, potentially enhancing treatment effectiveness.

An integrative approach that includes proteomics, genomics, and metabolomics could provide a more comprehensive understanding of EFS pathogenesis and allow for the development of more precise and personalized treatment methods. Integrating data from various "omics" technologies may help identify new biomarkers and therapeutic targets, improving outcomes for women with EFS. Additionally, evaluating the long-term outcomes of new diagnostic and therapeutic strategies based on proteomic data is essential. It is necessary to study how changes in proteomic profiles affect IVF success in the long term and how they relate to other clinical parameters, such as neonatal health and pregnancy complication risks.

In conclusion, our study demonstrates that proteomic analysis of follicular fluid can provide valuable insights into EFS pathophysiology and IVF outcome prediction. The identified protein markers may improve diagnosis and support the development of new therapeutic approaches aimed at enhancing the chances of successful IVF outcomes in women with EFS. Personalized treatment approaches based on patients' proteomic profiles present a promising direction for significantly improving reproductive program outcomes and quality of life for women with this syndrome. Additionally, the use of an integrative approach that includes proteomics, genomics, and metabolomics can provide a more comprehensive understanding of EFS pathogenesis and enable the development of more precise and personalized treatment methods. Integrating data from various "omics" technologies may help identify new biomarkers and therapeutic targets, ultimately leading to improved outcomes for women with EFS.

Moreover, an important direction for future research is the assessment of long-term outcomes of new diagnostic and therapeutic strategies based on proteomic data. It

is necessary to investigate how changes in proteomic profiles impact IVF success in the long term and how these changes correlate with other clinical parameters, such as neonatal health and pregnancy complication risks. In conclusion, our study demonstrates that proteomic analysis of follicular fluid can provide valuable insights into the pathophysiology of EFS and help predict IVF outcomes. The identified protein markers may improve diagnostic capabilities and support the development of new therapeutic approaches aimed at increasing the likelihood of successful IVF outcomes for women with EFS. Personalized treatment based on patients' proteomic profiles represents a promising direction that could significantly enhance the results of reproductive programs and the quality of life for women suffering from this syndrome.

CONCLUSION

The findings of our study underscore the importance of proteomic analysis in understanding the pathophysiological mechanisms underlying Empty Follicle Syndrome and its potential in predicting IVF outcomes. The observed differences in protein profiles between women with EFS and the control group indicate specific molecular changes that could serve as biomarkers for diagnosing and predicting this syndrome. Proteomic analysis revealed significant changes in the levels of proteins related to oxidative stress, inflammation, and apoptosis. Specifically, in Group I, elevated levels of Caspase-3 and IL-6 suggest intensified oxidative stress and inflammatory processes. Reduced levels of anti-apoptotic proteins, such as Bcl-2 and Survivin-2B, in this group may indicate increased cell death, negatively affecting oocyte quality and IVF success. In Group II, age-related changes were identified, including increased levels of ApoA-1 and fibronectin, as well as elevated levels of Caspase-3 and IL-8. These changes may reflect the accumulation of age-related damage and heightened inflammatory processes, which may also negatively impact IVF outcomes. Thus, our study results indicate that proteomic profiles can serve as reliable biomarkers for predicting IVF outcomes in women with EFS. The identified protein markers associated with inflammation, oxidative stress, and apoptosis suggest potential mechanisms in EFS pathogenesis and could be used to develop new therapeutic strategies. A personalized approach to treating EFS patients, considering each woman's age-related and biochemical characteristics, may improve the likelihood of successful IVF

outcomes. Further studies are necessary to confirm our findings and develop more effective methods for diagnosing and treating EFS.

Ethics approval and consent to participate - All patients gave written informed consent to participate in the study.

Consent for publication - The study is valid, and recognition by the organization is not required. The author agrees to open publication

Availability of data and material - Available

Competing interests - No

Financing - No financial support has been provided for this work

Conflict of interests-The authors declare that there is no conflict of interest.

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PUCH FOLLIKULA SINDROMI MAVJUD AYOLLARDA EKU NATIJASINI BAHOLASHDA PROTEOMIK TAHLILINING O'RNI

Olimova K.J., Shukurov F.I., Jalolova G.S.

Toshkent tibbiyot akademiyasi

REZYUME

Dolzarbligi. Puch follikul sindromi (PFS) mavjud ayollarda EKU natijalarini prognozlash zamonaviy reproduktiv tibbiyotidagi dozarb muammolardan biridir.

Maqsad. Puch follikula sindromi (PFS) aniqlangan ayollarda ekstrakorporal urug'lantirish (EKU) natijalarini prognozlashda proteomik profillarning o'rnini baholash.

Material va usullar. Tadqiqotga anamnezida puch follikula sindromi bo'lgan 60 nafar ayol kiritildi. Bemorlar uch guruhga bo'lindi: I guruh — erta reproduktiv yoshdagi 30 nafar ayol (18-35 yosh), II guruh — kech reproduktiv yoshdagi 30 nafar ayol (36-41 yosh), nazorat guruhi — reproduktiv muammosiz sog'lom ayol tashkil etdi.

Natijalar. Ushbu guruhda Bcl-2 va Survivin-2B kabi antiapoptotik oqsillar darajasining pasayishi hujayralarning ko'payib nobud bo'lishiga ishora qilishi mumkin, bu esa ootsitlar sifati va EKU muvaffaqiyatiga salbiy ta'sir qilishi mumkin. II guruhdagi ayollarda yoshga bog'liq jarayonlarga oid o'zgarishlar, jumladan, ApoA-1 va fibronektin darajalari oshishi, shuningdek, Kaspaza-3 va IL-8 darajalari oshishi kuzatildi. Xulosa. Tadqiqot natijalari proteomik profillar PFS bo'lgan ayollarda EKU muvaffaqiyatini bashorat qilish uchun ishonchli biomarker bo'lishi mumkinligini ko'rsatdi. Yallig'lanish, oksidlovchi stress va apoptoz bilan bog'liq aniqlangan belok markerlari PFS patogenezini mexanizmlarini tushinishga ishora qilib, yangi terapevtik strategiyalarni ishlab chiqishda foydalanilishi mumkin. Shu tariqa, olingan ma'lumotlar PFS bo'lgan bemorlarga yosh va biokimyoviy xususiyatlarini inobatga olgan holda individual yondashuv muhimligini ta'kidlaydi, bu esa EKU muvaffaqiyati ehtimolini oshirishi mumkin.

Kalit so'zlar: proteomika, ekstrakorporal urug'lantirish, bosh follikula sindromi, biomarkerlar, prognozlash, reproduktiv tibbiyot.

РОЛЬ ПРОТЕОМИЧЕСКОГО АНАЛИЗА В ПРОГНОЗИРОВАНИИ ИСХОДА ЭКО У ЖЕНЩИН С СИНДРОМОМ ПУСТЫХ Фолликулов

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РЕЗЮМЕ

Актуальность. Прогнозирование результатов ЭКО у женщин с синдромом пустых фолликулов (СПФ) является одной из критических задач современной репродуктивной медицины.

Цель. Оценка роли протеомических профилей в прогнозировании исходов экстракорпорального оплодотворения у женщин с синдромом пустых фолликулов.

Материал и методы. В исследование было включено 60 женщин с синдромом пустых фолликулов (СПФ) в анамнезе.

Результаты. Сниженные уровни антиапоптотических белков, таких как Bcl-2 и Survivin-2B, в этой группе могут свидетельствовать о повышенной клеточной гибели, что может отрицательно сказываться на качестве ооцитов и успехе ЭКО. У женщин из II группы были выявлены изменения, связанные с возрастными процессами, включая повышение уровней ApoA-1 и фибронектина, а также повышение уровней Каспаза-3 и IL-8.

Заключение. Результаты исследования показали, что протеомические профили могут служить надежными биомаркерами для прогнозирования успеха ЭКО у женщин с СПФ. Обнаруженные белковые маркеры, связанные с воспалением, окислительным стрессом и апоптозом, указывают на возможные механизмы патогенеза СПФ и могут быть использованы для разработки новых терапевтических стратегий. Таким образом, полученные данные подчеркивают важность индивидуального подхода к лечению пациенток с СПФ, учитывающего возрастные и биохимические особенности каждой женщины, что может повысить вероятность успешного исхода ЭКО.

Ключевые слова: протеомика, экстракорпоральное оплодотворение, синдром пустых фолликулов, биомаркеры, прогноз, репродуктивная медицина