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Improving Methods For Diagnosing Endocrine Infertility

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Abstract: Endocrine infertility is one of the most common forms, the origin of which is directly related to a decrease in ovarian reserve, hormonal imbalance, metabolic changes, and immunological factors. Functional disorders in the endocrine system disrupt the maturation process of egg cells, cause ovulation to fail, and this causes serious problems in restoring fertility in women

Objective: In the early diagnosis and prognosis of women with endocrine infertility, the level of expression of epigenetic markers Let-7b-5p, miR-223-3p, and miR-320a was studied, and their pathogenetic relationship with clinical, hormonal, and echographic indicators was proven.

Results: This study is distinguished by the fact that it covers biomarker-based approaches in the diagnosis of women with infertility of endocrine genesis, and this algorithm is important for restoring fertility and improving the resultsThe introduction of differentiated, pathogenetically based, and individualized approaches to cases of infertility of endocrine origin not only increased clinical effectiveness, but also provided a wide range of social and economic benefits. According to the results of the study, in women with infertility against the background of hyperprolactinemia, hypothyroidism, and ovarian dysfunction, against the background of targeted therapy (cabergoline, tyramine, ovariamine), the frequency of ovulation and pregnancy significantly increased (56.7-66.7% and 33.3-40.0%, respectively).

Conclusions:The introduction of differentiated, pathogenetically based, and individualized approaches to cases of infertility of endocrine origin not only increased clinical effectiveness, but also provided a wide range of social and economic benefits.

this study is distinguished by the fact that the diagnosis of women with infertility of endocrine genesis includes individual, biomarker-based approaches, which is important for the restoration of fertility and improvement of results.

Keywords: Infertility, endocrine infertility, pituitary dysfunction, thyroid dysfunction, and 30 ovulatory dysfunction.

Introduction: Infertility is one of the most common reproductive problems among women in the world. According to the World Health Organization (WHO), the incidence of infertility is currently 10-13%.(1) Large-scale scientific research is being conducted worldwide to identify and effectively treat infertility of endocrine origin. Accurate diagnosis based on modern biomarkers, personalized hormone therapy, genetic analysis, and metabolomic methods, as well as the development of individual treatment plans for each patient, has become one of the most pressing areas in this area. (8) At the same time, there are a number of problems that need to be solved in this area: increasing the sensitivity and accuracy of diagnostics, introducing innovative therapeutic methods, and further improving the personalized approach to patients. This indicates a high need for new scientific solutions and practical approaches in the field. (2)

Consistent and comprehensive reforms are being carried out in our country to develop the medical sphere, bring the healthcare system in line with world standards, improve the quality of medical services provided to the population, and expand the coverage of medical care.(7) As one of the priority areas of state policy aimed at radically improving the healthcare system, measures are being implemented aimed at "bringing primary healthcare services closer to the population, protecting motherhood and childhood,

preserving reproductive health and meeting needs, expanding the scope of specialized medical care."(3)

It is in these processes that research and practical solutions in the direction of early detection of endocrine infertility, effective diagnosis of reproductive disorders associated with ovulatory dysfunction, hormonal imbalance, and decreased ovarian reserve in women, as well as improvement of treatment based on an individual approach, are of particular importance. (4)This, in turn, serves to increase the effectiveness of the healthcare system by preserving the reproductive potential of the population, restoring fertility, and eliminating the endocrine causes of infertility.(5)

The purpose of the study is to assess the state of epigenetic markers in women with infertility of various endocrine origins and their diagnostic and prognostic significance;(6)

METHODS

A total of 320 women of reproductive age were included in the study, of which a retrospective analysis of the incidence of endocrine infertility was conducted in 200 of them. The prospective study included 120 women of reproductive age, who were divided into three main groups: group I consisted of 30 infertile women with pituitary dysfunction (hyperprolactemia), group II consisted of 30 infertile women with thyroid dysfunction (hypothyroidism), group III consisted of 30 infertile women with ovulatory dysfunction (ovarian polycystic syndrome), and the control group consisted of 30 healthy women of reproductive age. During the study, immunological, echodopplerometric, hormonal studies, and studies aimed at determining ovarian reserve were conducted in women.

The study was carried out in several stages: At the first stage (2020-2023), the results of the study were presented. A retrospective analysis of the incidence of endocrine infertility in 200 women of reproductive age was conducted.

In the second stage (2022-2025), clinical and laboratory studies were conducted in 30 women with infertility associated with pituitary dysfunction, 30 with thyroid dysfunction, and 30 with ovulatory dysfunction (ovarian polycystic syndrome), including hormonal, immunological, echodopplerometric, and prospective studies aimed at determining ovarian reserve.

In the third stage, studies were conducted to determine the state of the epigenetic markers let-7b-5p, miR-223-3p, and miR-320a before and after drug therapy in 30 women with infertility associated with pituitary dysfunction, 30 with thyroid dysfunction, and 30 with ovulatory dysfunction, as well as the effectiveness of treatment outcomes.All women, the conducted studies met the inclusion criteria, and no diseases associated with the exclusion criteria were identified.

In women with endocrine infertility, the level of expression (fold change) of epigenetic markers let-7b-5p, miR-223-3p, and miR-320a, the circulatory cfDNA methylation index (%), and histone modifications (ng/mg histone protein) were determined.

A serum sample (5 ml) from venous blood was used as biological material. Blood was centrifuged at a rate of 3000 g for 10 minutes, serum was isolated, and stored at a temperature of -80°C. Isolation of markers from serum was carried out using the reagent TrizolTM (Invitrogen, USA). Trizol reagent was added to 200 µl of serum, and RNA was purified by phase separation with chloroform, precipitation with isopropanol, and washing with 75% ethanol during vigorous stirring. Based on the obtained markers, the synthesis of complementary DNA (cDNA) was carried out using miScript II RT Kit (Qiagen, Germany).

Real-time PCR analysis was performed using the QuantStudioTM 5 Real-Time PCR system (Thermo Fisher Scientific, USA) and the miScript SYBR Green PCR Kit (Qiagen). For each marker, special primers (Qiagen custom-designed primers) were used. For normalization of the expression level, U6 small nuclear RNA (U6 snRNA) was selected as internal control. PCR thermoprofile: 15 seconds at 95°C, 30 seconds at 60°C - 40 cycles. The relative degree of expression was calculated by the method ΔCt and ΔΔCt (based on the formula 2^-ΔΔCt). All analyses were performed at least in duplicate.

To determine the cfDNA methylation index (%), sodium bisulfite conversion on cfDNA isolated from serum (Zymo Research, USA) and methyl-specific PCR (MSP) were used. The level of methylation was calculated using methylated and non-methylated primers specific to the markers, and the overall index (%) was formed.

To determine the level of histone modifications (ng/mg histone protein), special histone extraction kits (Abcam, UK) and ELISA enzyme-linked immunosorbent assay were used. In each sample, the absolute content of epigenetic markers let-7b-5p, miR-223-3p, and miR-320a, associated with histone modifications, was calculated.

All data obtained as a result of the analysis were statistically processed using the GraphPad Prism 9.0 and SPSS 26.0 programs. The difference between the groups was assessed using the t-test, Mann-Whitney, ANOVA, and correlation (Pearson/Spearman) methods.

To ensure the reliability of the research results, modern statistical methods were used to analyze clinical,

hormonal, and instrumental indicators in women with endocrine infertility. For statistical processing and analysis of all data, the IBM SPSS Statistics 28.0 program was used, which ensured the accuracy and reliability of the analysis process.

Fisher's criterion and the Kolmogorov-Smirnov test were used to assess the distribution of data. When comparing quantitative indicators between groups, Student's t-test and variance analysis (ANOVA) were used for parametric data, and Mann-Whitney and Wilkinson criteria were used for non-parametric data. When comparing categorical indicators between groups, χ2 and Fisher accuracy criteria were used.

Spearman's rank correlation coefficient was calculated to determine the degree of correlation and influence between the indicators. The reliability of the correlation was assessed using the t-criterion. Logistic regression, ROC analysis, and variation analysis were also used to assess the prognostic and diagnostic models in the study, as well as to predict the effectiveness of treatment. In ROC analysis, "AUC" (Area Under Curve) and their confidence intervals were calculated for the indicators.

In statistical analysis, the differences were considered significant at the level of $p<0.05$; $p<0.01$ and $p<0.001$.

The obtained results were reflected in tables and diagrams, analyzed in detail in the research sections, and the main conclusions were drawn.

Within the framework of the study, 90 women with infertility were divided into four groups based on etiological criteria: women with infertility associated with pituitary dysfunction (n=30), women with infertility associated with thyroid dysfunction (n=30), women with infertility associated with ovulatory dysfunction (n=30), and the control group consisted of women (n=30).

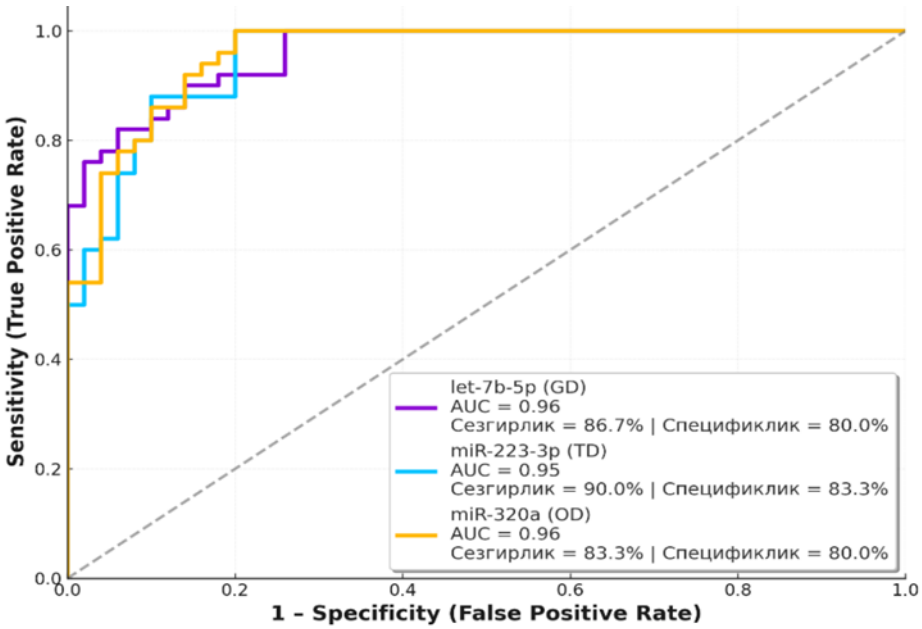
In all participants, the expression of epigenetic markers let-7b-5p, miR-223-3p, and miR-320a in blood serum (fold change), cfDNA methylation index (%), and histone modifications (ng/mg histone protein) were determined.

According to the analysis results, let-7b-5p expression in women of group I was significantly reduced compared to the control (0.47±0.015; $p<0.001$), and the cfDNA methylation index was characterized by pronounced hypermethylation (78.3±2.6%; in the control 49.1±1.6%, $p<0.001$).Among histone modifications, the level of H3K27me3 was elevated (6.1±0.21 ng/mg; in the control 3.5±0.12 ng/mg), while H3K4me3 was decreased (2.6±0.29 ng/mg; in the control 5.8±0.20 ng/mg; $p<0.001$).

In women of group II, the expression of miR-223-3p was highest (2.35±0.08; $p<0.001$), cfDNA methylation was low (42.7±1.4%), histone modifications H3K9ac (7.4±0.25 ng/mg) and H3K27me3 (4.5±0.16 ng/mg) were significantly increased ($p<0.001$).

In women of group III, the expression of miR-320a was reduced (0.58±0.019; $p<0.001$), cfDNA methylation was high (73.2±2.5%; $p<0.001$), H3K4me3 and H3K27ac levels were low (2.2±0.075 and 1.5±0.049 ng/mg).

It was established that the indicators of epigenetic markers have high diagnostic value according to ROC analysis. In particular, ROC analysis for the let-7b-5p cfDNA methylation index in the diagnosis of pituitary dysfunction showed the possibility of accurate diagnosis with AUC = 0.89 (95% CI: 0.82-0.96), sensitivity - 86.7%, specificity - 80.0%.For the expression of miR-223-3p in thyroid dysfunction, AUC = 0.93 (95% CI: 0.87-0.98), sensitivity - 90.0%, specificity - 83.3%. In the miR-320a cfDNA methylation index for ovulatory dysfunction, AUC = 0.87 (95% CI: 0.80-0.95), sensitivity - 83.3%, specificity - 80.0% were noted.



Results of ROC analysis of epigenetic markers

The study also analyzed the correlation between the expression of epigenetic markers let-7b-5p, miR-223-3p, miR-320a, cfDNA methylation, histone modifications, and clinical and hormonal indicators: in pituitary dysfunction, let-7b-5p methylation and prolactin levels were determined at r=0.62 (p<0.01), in thyroid dysfunction, miR-223-3p expression and TSH, in ovulatory dysfunction, miR-320a expression and AMH were determined at r=0.55 (p<0.05) (Fig. 3.14).

In general, the results of this epigenetic study showed the high modern diagnostic and prognostic value of the biomarker let-7b-5p, miR-223-3p, and miR-320a in the early detection of types of endocrine infertility and the determination of personalized therapy tactics.

Based on these results, epigenetic markers can be used as the main molecular-pathogenetic agent in the early detection, risk assessment, and personalized treatment strategy for infertility against the background of endocrine diseases. In addition, it turned out that these biomarkers have high potential for assessing and predicting reproductive function in clinical practice.

Within the framework of the study, in order to expand the possibilities of pathogenetically differentiating and accurately diagnosing types of infertility of endocrine genesis, the levels of epigenetic markers were analyzed. Notably, let-7b-5p levels were significantly elevated in cases of hyperprolactinemia, miR-223-3p levels in hypothyroidism, and miR-320a markers in cases of ovulatory dysfunction.

These results make it possible to effectively use molecular biomarkers in the formation of a personalized reproductive approach, early diagnosis, and assessment of the probability of restoring fertility in women with endocrine diseases. This approach, based on indicators of the level of expression of epigniotic markers, can serve as a reliable diagnostic and prognostic platform for further implementation into clinical practice.(10-15)

Also, in this study, an algorithm for the diagnosis and treatment of women with endocrine infertility was developed, which is distinguished by the fact that it includes individual, biomarker-based approaches, which is important for restoring fertility and improving the results.

CONCLUSION

It has been proven that the expression of epigenetic markers let-7b-5p, miR-223-3p, and miR-320a, methylation of cfDNA, and changes in histone modifications in women with endocrine infertility have high diagnostic and prognostic value in the early detection of infertility, individual assessment of fertility recovery, and the choice of personalized treatment methods, and the logistic regression model developed on the basis of these markers is effective in the diagnosis, prediction, and treatment of endocrine infertility (AUC = 0.958; sensitivity - 92.0%; specificity - 89.5%).

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