ABSTRACT

On the dissertation of Rybina Anastassiya Nikolayevna,
entitled "A Personalized Approach to Overcoming Infertility in Women of Advanced Reproductive Age" submitted for the degree of Doctor of Philosophy (PhD) 8D10103 – Medicine PhD Dissertation

Relevance of the research topic

Infertility is defined as the absence of pregnancy for 12 months in a couple of reproductive age who do not use contraception and have regular sexual intercourse. According to published data, the prevalence of infertility ranges from 15–18% and shows no tendency to decrease. In Kazakhstan, according to the literature, the prevalence of infertility reaches 20% and likewise does not decline. At present, the use of assisted reproductive technologies (ART) is the most effective method of overcoming infertility.

Despite the rapid development of assisted reproductive technologies and the emergence of new methods, the effectiveness of ART programs varies across countries but generally remains stable. The main reason for unsuccessful outcomes is the advanced reproductive age of patients seeking treatment in reproductive clinics. It is well known that fertility begins to decline as early as the age of 30, with a more pronounced decline after 37 years. This is supported by numerous publications, reports of international professional associations, and data from the Kazakhstan Association of Reproductive Medicine.

The successful outcome of ART procedures depends on multiple factors, including the causes of infertility, the age of the partners, and the quantity and quality of embryos.

Controlled ovarian stimulation (COS) is a key stage of ART. Personalization of gonadotropin dosing is based on the assessment of genetic markers, including polymorphisms of the FSH receptor gene (rs6166) associated with sensitivity to follicle-stimulating hormone. Genes involved in folate metabolism also play an important role, influencing ovarian response, embryo development, and embryo quality.

Implantation depends not only on embryo quality but also on endometrial receptivity. Chronic endometritis is detected in 10–15% of patients and in up to 42% of women with recurrent IVF failures. Modern diagnostic methods, including immunohistochemistry, increase detection rates; however, treatment remains challenging and may require repeated therapeutic courses.

Thus, women of advanced reproductive age require a personalized approach to overcoming infertility, consisting of comprehensive preparation for ART programs, including genotypic preconception preparation, evaluation and treatment of endometrial pathology, embryo assessment, and transfer of euploid embryos only.

The aim of the study was to improve the effectiveness of assisted reproductive technology programs by optimizing preparation in women of advanced reproductive age of the Kazakh population based on a personalized approach.

Study objectives included:
1. To investigate the structure and effectiveness of ART programs in the Republic of Kazakhstan depending on female age;
2. To study the prevalence of allele and genotype polymorphisms of folate metabolism genes MTHFR rs1801133 and rs1801131, MTR rs1805087, MTRR rs1801394 and FSH receptor genes rs6165 and rs6166, and to assess the impact of unfavorable genotypes on ART outcomes in infertile women aged ≥35 years in the Kazakh population;
3. To evaluate endometrial status and the prevalence of endometrial pathology in women of advanced reproductive age (≥35 years);
4. To assess the morphological, morphokinetic, and genetic characteristics of embryos using aCGH in ART programs in women aged ≥35 years;
5. To evaluate the effectiveness of ART programs using a personalized approach in women of advanced reproductive age of the Kazakh population.

Study design: investigator-initiated research conducted as part of a PhD dissertation.

Research methods

Clinical and anamnestic (questionnaires, general clinical and laboratory examination)

A retrospective analysis of the medical records of 150 Kazakh female infertility patients was conducted.

A prospective clinical and anamnestic examination was conducted on 69 Kazakh female infertility patients who had undergone assisted reproductive technology (ART) programs – 30 patients in the study group and 39 patients in the comparison group. Standard clinical and laboratory examinations were conducted in accordance with Order No. 172 of the Ministry of Health of the Republic of Kazakhstan.

Genetic methods: real-time PCR determination of *FSHR rs 6165* and *rs 6166* gene polymorphisms; *MTHFR rs1801133 (C677T), rs1801131 (A1298C), MTR rs1805087 (A2756G), MTRR rs1801394 (A66G)* were analyzed by PCR. A total of 150 *FSHR rs6165* and *rs6166* gene assays were performed in a retrospective group. A total of 150 PCR assays were performed for *MTHFR rs1801133 (C677T), rs1801131 (A1298C), MTR rs1805087 (A2756G), MTRR rs1801394 (A66G).*

Thirty studies of the *MTHFR rs1801133 (C677T), rs1801131 (A1298C), MTR rs1805087 (A2756G), and MTRR rs1801394 (A66G)* genes were conducted using the PCR method in a prospective group.

Chromosomal microarray analysis (aCGH) of embryonic trophectoderm. A total of 262 aCGH results of embryonic trophectoderm were analyzed retrospectively. Thirty aCGH of embryonic trophectoderm were prospectively performed.

Hysteroscopy, endometrial biopsy, histological examination, and immunohistochemical study of CD138. A retrospective analysis of 569 office hysteroscopy protocols and the results of histological and histochemical examinations was conducted. A total of 45 office hysteroscopies with histological and histochemical examination of the endometrium were prospectively performed.

Statistical methods. The following methods were used: descriptive statistics (mean, standard deviation, median, interquartile range); Pearson's χ^2 test for qualitative analysis; Student's t-test for comparison of quantitative parameters; Pearson correlation analysis; logistic regression; classification trees, odds ratios (OR), and 95% confidence intervals. Differences were considered statistically significant at $p < 0.05$.

The study was approved by the Local Ethics Committee of the International Clinical Center of Reproduction PERSONA LLC No. 2 dated November 23, 2020, and the Local Ethics Committee of the Kazakh National Medical University No. 5 (111) dated April 28, 2021. All patients received full information about the purpose of the study and signed voluntary informed consent.

Objects and subjects of the Study:

Retrospective part of the study:

Stage 1 — analytical.

An analysis of the structure of IVF and embryo transfer programs in the Republic of Kazakhstan was conducted:

• data from the National ART Registry for 2020–2022

• data from the PERSONA Clinic for 2020–2024

The total number of cycles analyzed was 8,611.

Stage 2 — population genetic analysis. The frequency of gene polymorphisms was determined for the following genes: FSHR, MTHFR, MTR, and MTRR in the Kazakh population.

The study included 150 patients undergoing ART programs.

Stage 3 — clinical and embryological analysis. The study included:

• 569 hysteroscopies in women with infertility

• 1,082 embryo transfers:

• 387 in women aged 35 years or older

• 695 in women aged <35 years

• A study of 431 single embryo transfers with morphokinetics analysis was also conducted.

Prospective part of the study:

Stage 4 – prospective cohort study.

The effectiveness of a personalized preparation algorithm was assessed, with the formation of a study group (30 patients) and a control group (39 patients).

All patients were selected according to the following criteria.

Inclusion criteria for the retrospective study:

• Kazakh ethnicity through maternal and paternal grandparents, according to a survey, and a history of unsuccessful ART programs.

• Exclusion criteria:

• Spermatogenesis disorders, endometriosis based on ultrasound, carriage of balanced chromosomal abnormalities based on karyotyping of both spouses, developmental anomalies and pathologies of the internal genital organs that prevent pregnancy, donor eggs, surrogacy.

To test the algorithm for personalized patient care for women of advanced reproductive age in the Kazakh population, a prospective cohort study was conducted from January 2024 to December 2024. The main group included 30 patients who underwent personalized pre-pregnancy preparation, and the control group included 39 patients who underwent standard examination and preparation before the IVF and ET program.

Inclusion criteria for the main group:
• Membership of the Kazakh population
• Personalized preconception preparation
• Age ≥35 years

Inclusion criteria for the control group:
• Membership of the Kazakh population
• Standard preconception preparation
• Age ≥35 years
• Fresh embryo transfer within 5 days

Exclusion criteria for the study:
• Age <35 years
• Membership of a non-Kazakh population
• Severe spermatogenesis disorders in the man
• Congenital genital anomalies that prevent pregnancy
• Uterine fibroids, endometriosis that prevent pregnancy
• Karyotype changes in one/both spouses
• Donor oocytes
• Surrogacy.

Study subject: A Kazakh woman with infertility. Subject of the study:

The effectiveness of assisted reproductive technology programs in women of advanced reproductive age in the Kazakh population based on a personalized approach to preconception preparation using genotyping, lifestyle modification, uterine examination and treatment, personalized controlled ovarian stimulation, and embryo morphokinetics and ploidy studies.

The main theses of the dissertation submitted for defense:

1. Patients of advanced reproductive age (≥35 years) account for 49-58% of all women seeking ART treatment. Overcoming infertility in this group requires a personalized approach.

2. The carriage of unfavorable polymorphisms of the rs1801133 and rs1801131 gene alleles and genotypes of the MTHFR gene, rs1805087 gene MTR, and rs1801394 gene MTRR in the Kazakh population of infertile women reaches 90% and is associated with adverse outcomes in ART programs. Screening for folate metabolism gene polymorphisms and genotypic preconception preparation are clinically justified. The carriage frequency of the FSHR gene allele polymorphism and genotypes rs6165 and

rs6166 in the Kazakh population is not different from that in global populations. Carriage of the minor allele does not affect the outcome of ART programs.

3. Chronic endometritis was detected in 85% of women of advanced reproductive age (≥35 years), with severe chronic endometritis diagnosed in 12.74% of women ≥35 years with infertility. Advanced reproductive age in infertile patients requires mandatory uterine examination by hysteroscopy with endometrial biopsy and IHC for CD138.

4. A woman's age negatively impacts the morphological, morphokinetic, and genetic characteristics of embryos. Evaluation of the morphokinetics of AI and PGT-A embryos and euploid embryo transfer can increase pregnancy and live birth rates and reduce pregnancy loss.

5. Personalized pre-conception preparation for the ART program significantly reduces the time to achieve pregnancy, increases the frequency of live births and reduces pregnancy losses.

Description of the Main Study Results

A retrospective analysis of ART programs conducted at the PERSONA International Clinical Center for Reproductive Medicine from 2020 to 2024 showed that women of advanced reproductive age accounted for 62.37% of all 8,611 cycles. The pregnancy rate after age 35 declines, reaching 0% after age 44.

Data from the National ART Registry for 2020-2022 indicate an increase in ART programs in the Republic from 17,743 to 26,800. The proportion of women 35 and older also increased to 49%. However, pregnancy and live birth rates remain low, reaching 0% by age 44.

Our study found that carriage of unfavorable alleles of folate metabolism genes (MTHFR, MTR, MTRR) is statistically significantly associated with the number of ART programs, the duration of infertility, and the incidence of pregnancy loss. Thus, carriage of the A/G and G/G alleles of the MTRR gene is associated with an increased incidence of embryo aneuploidy and reduced embryo quality.

The carriage rate of minor alleles of the FSHR genes (rs6166, rs6165) in the Kazakh population does not significantly differ from that of European and South Asian populations, but is higher than that of East Asian populations. This underscores the importance of accounting for population differences in genetic studies. In our study, there was no statistically significant difference in the outcomes of female stimulation depending on the carriage of the FSHR rs6166 and rs6165 genotypes and alleles. Therefore, we do not recommend this test as routine testing before initiating IVF and ET programs.

A woman's age influences embryo quality. After age 35, the likelihood of obtaining embryos of low morphological quality is higher than before age 35. Embryos of low morphological quality are more likely to implant in younger women than in older reproductive age women. The probability of implantation and pregnancy is comparable when transferring both morphologically "good" and "suboptimal" embryos. Embryos of average quality have the same implantation potential as embryos of good quality.

There are differences in morphokinetics: embryos from older reproductive age women have embryos with a lower AI development score; to achieve pregnancy in older

reproductive age, embryos with a higher AI score are required. Even morphologically high-quality embryos in older women can be genetically abnormal.

In women of older reproductive age, the number of euploid embryos decreases with increasing age. Correlation analysis showed a negative association between a woman's age and the number of genetically normal embryos (r = -0.797). Pregnancy rates are significantly higher with the transfer of embryos that have undergone PGT-A: 80% for high-quality embryos versus 35.6% in the group without testing.

Morphological assessment of mid- and low-quality embryos does not always correlate with their genetic status, confirming the need for genetic screening.

Based on the data obtained, an algorithm for personalized preparation for the ART program was developed: Examination and treatment are conducted 1-2 months prior to the ART program and include lifestyle modification, BMI normalization, and the following:

1. Preparation for ART: genotyping for the carriage of gene allele polymorphisms and genotypes of rs1801133 and rs1801131 of the MTHFR gene, rs1805087 of the MTR gene, and rs1801394 of the MTRR gene. Genotypic folate doses: high-risk group (homozygotes and heterozygotes for the unfavorable allele of the MTHFR gene) - 5 mg of folic acid, low-risk group (homozygotes for the favorable allele of the MTHFR gene) - 400 mcg of folic acid. Carriers of unfavorable alleles of the MTR and MTRR genes should additionally receive vitamin B12 supplements – 2.6 mcg.

2. Hysteroscopy with endometrial biopsy and immunohistochemistry, treatment of chronic endometritis.

3. Initiation of ovarian-associated ovarian stimulation (OAS) with basal FSH levels ≤9.3 IU/L, including LH supplementation. If basal FSH levels are ≥9.3 IU/L, estrogen priming should be administered to lower FSH levels.

4. Embryo cultivation in a TimeLapse incubator, evaluation of the IU/IU, and embryo selection for PGT-A.

5. PGT-A.

6. Cryotransfer of a euploid embryo (Figure 1).

Patients who underwent personalized preparation, including genotyping, hysteroscopy, and treatment of chronic endometritis, had better ART program outcomes. The live birth rate in the study group was 53.33%, which was significantly higher than in the standard group, where this rate was 17.95% (p = 0.008). Furthermore, the pregnancy loss rate in the study group was statistically significantly lower—15.8% versus 41.67% in the control group (p = 0.022), demonstrating the superiority of a personalized approach in reducing the risk of adverse reproductive outcomes in patients of advanced reproductive age.

Scientific Novelty:

1. The carriage frequency of the FSH rs6165 and rs6166 alleles and genotypes was determined for the first time in the Kazakh population;

2. The carriage frequency of the MTHFR rs1801133 (C677T), rs1801131 (A1298C), MTR rs1805087 (A2756G), and MTRR rs1801394 (A66G) alleles and genotypes was determined for the first time in Kazakh women with infertility;

3. Associations between genetic and clinical factors were established;

4. An algorithm for personalized preparation of older reproductive-age patients for ART was developed.

Practical significance of the obtained results

1. Reducing the time to pregnancy:

A personalized approach to preparing patients for ART (assisted reproductive technology) programs takes into account individual reproductive health, hormonal levels, and ovarian response, which facilitates faster and more effective embryo implantation.

2. Improving the pregnancy rate and outcome:

Individualizing the ovulation stimulation protocol, embryo selection, and transfer timing increases the likelihood of clinical pregnancy and, consequently, increases the live birth rate.

3. Reducing the risk of reproductive loss:

By more accurately assessing factors influencing implantation and early gestation, a personalized approach reduces the incidence of early miscarriages, biochemical pregnancies, and other forms of loss.

4. Optimizing the overall reproductive prognosis:

Individualizing the preparation regimen improves treatment tolerability, reduces the number of unsuccessful ART cycles, and increases the chances of successfully completing the program with the birth of a healthy child.

Doctoral student's personal contribution: Developing the research goals and objectives, collecting data and examining patients, directly analyzing outpatient records, statistically processing the research results, and formulating conclusions and recommendations.

Conclusions:

1. Women of advanced reproductive age account for 49-58% of all patients seeking ART in Kazakhstan. The pregnancy and birth rate after age 35 significantly decreases and approaches zero after age 44.

2. The carriage of unfavorable polymorphisms of the gene alleles and genotypes rs1801133 and rs1801131 of the MTHFR gene, rs1805087 of the MTR gene, and rs1801394 of the MTRR gene in the Kazakh population of infertile women reaches 90% and is associated with negative outcomes in ART programs. In the presence of 4 polymorphisms, the aneuploidy rate reaches 63.64%.

The carriage frequency of the FSHR gene allele polymorphism and genotypes rs6165 and rs6166 in the Kazakh population is not different from that in global populations. Carriage of the minor allele does not affect the outcome of ART programs.

3. Severe chronic endometritis in women of advanced reproductive age is twice as common as in women under 35. Advanced reproductive age in infertility patients is an

indication for uterine examination by hysteroscopy with endometrial biopsy and immunohistochemistry for CD138.

4. In women of advanced reproductive age, the risk of obtaining embryos of good morphological quality is 16.3% lower, while the risk of obtaining embryos of poor quality is 22.6%. In women over 35, embryo morphokinetics is characterized by a 16.8% decrease in the frequency of embryos with a high developmental score according to artificial intelligence. In women over 35, 69% of embryos are aneuploid. Evaluation of the morphokinetics of AI and PGT-A embryos and the transfer of euploid embryos can increase pregnancy and live birth rates and reduce pregnancy loss.

5. Implementation of a personalized preconception preparation algorithm for ART programs doubles the pregnancy rate, increases the live birth rate by 2.9 times, and reduces pregnancy loss by 2.6 times.

Dissertation Results Approval

The main research results were presented at international and national conferences:

- International Scientific and Educational Congress dedicated to the 50th Anniversary of the Scientific Center of Obstetrics, Gynecology, and Perinatology, May 14-16, 2025;

- International Congress of the Russian Academy of Sciences "Age and Reproduction: Innovations in Reproductive Medicine," June 10, 2025.

- Extended Meeting of the Department of Obstetrics and Gynecology of the Kazakh National Medical University named after S.D. Asfendiyarov, No. 6, June 25, 2025.

Publications on the Dissertation Topic

Seven papers based on the research materials have been published, including two articles in non-CIS journals indexed by Web of Knowledge (Thomson Reuters, USA) and Scopus (Elsevier, Netherlands). 5 articles in the journal of the Republic of Kazakhstan, included in the list of those recommended by the Control Committee of the Ministry of Education and Science of the Republic of Kazakhstan; 2 practical guidelines; 1 clinical protocol of the Ministry of Health of the Republic of Kazakhstan.

Number of published articles by quartile - 2:

Q3% (35%) – 1:

Lokshin V.N., Rybina A.N., Abshekenova A.T., Askar E., Karibaeva Sh.K., Valiev R.K. Uterine cavity condition in patients of advanced reproductive age. Obstetrics, Gynecology and Reproduction. 2025;19(4):506–513. https://doi.org/10.17749/2313-7347/ob.gyn.rep.2025.654.

Q2% (60%) – 1:

Lokshin V, Askar Y, Rybina A., Abshekenova A, Karibayeva Sh, Valiev R, Saduakas A. Antibiotics and Uterine Flushing versus Antibiotics Alone for Chronic Endometritis with Thin Endometrium in Assisted Reproductive Technology: A Single-Center Retrospective Cohort Study. International Journal of Fertility and Sterility 2025; 19(2): 186-192. doi: 10.22074/ijfs.2024.2014586.1565

Number of published articles by KOKSNVO – 5:

Rybina A.N., A. Ellenbogen, D.D. Mukushkina, Sh.K. Karibayeva, R.K. Valiev. Outcomes of ART programs depending on the status of folate metabolism genes in the Kazakh population. No. 1 2024 Reproductive medicine (Central Asia), 17-26. https://doi.org/10.37800/RM.1.2024.17-26.

A.A. Begimbaeva, Rybina A.N., K.T. Nigmetova, Zh.K. Sailau, A.Sh. Ermekova, Sh.K. Karibaeva, V.N. Lokshin. Artificial intelligence is the key to the development of the embryology laboratory. No. 3 (2024) Reproductive medicine (Central Asia). DOI: https://doi.org/10.37800/RM.3.2024.42-49.

Rybina A.N., A.B. Kemel, R.K. Valiev, E. Askar, G.A. Tanysheva, A. Ellenbogen. Modern methods of managing patients with poor ovarian response to controlled ovarian stimulation: a literature review. No. 4 (2024): Reproductive Medicine (Central Asia). DOI: https://doi.org/10.37800/RM.4.2024.283.

Rybina, A.N., Abshekenova, A.T., Askar, E., Karibaeva, Sh.K., Valiev, R.K. Efficiency of a personalized algorithm for preparing patients of advanced reproductive age for an IVF program: a prospective cohort study. Reproductive Medicine (Central Asia) No. 2 (2025), 92–101/

DOI: https://doi.org/10.37800/RM.2.2025.535

Rybina, A.N., Valiev, R.K., Kemel, A., Askar, E., Bishchekova, B.N. Comparison of the efficacy and safety of hMG and r-FSH+LH preparations deriving their biological activity from different LH sources: a single-center cohort study. Reproductive Medicine (Central Asia), (2), 107–111. https://doi.org/10.37800/RM.2.2025.476

Dissertation Length and Structure

The dissertation is 108 pages long and consists of an introduction, literature review, research materials and methods, three main sections, a conclusion, and a list of references. The work is illustrated with 31 tables and 42 figures. The bibliography includes 187 sources.