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# PERSONIFIED APPROACH TO GENETIC SCREENING OF INFERTILITY COUPLES IN ART PROGRAMS (literature review)

**Abstract.** Infertility isan inability of a person of childbearing age to reproduce the offspring and it represents a serious medical and social problem. In recent years, the assisted reproductive methods of technology (further - ART) are increasingly being used to treat infertility, but despite effective advances in the treatment of infertility by ART, the problem of a birth of healthy offspring remains relevant.

Identification of genetic causes of infertility in couples significantly increases the effectiveness of ART: increases the frequency of implantation and the frequency of pregnancy, reduces the incidence of miscarriage and the risk of birth with offspring with chromosomal pathology and congenital malformations.

Methods: The analysis of modern literature data confirming the importance of genetic research in the diagnosis and treatment of infertility is carried out.

Results: In the presented literature review, the necessity of conducting medical genetic counseling services of couples with infertility with obligatory cytogenetic investigation of both spouses, the molecular genetic study of men with pathospermia and mandatory preimplantation diagnosis of embryos due to the high risk of transmission of chromosomal pathology to offspring was demonstrated. It significantly changes the modern tactics of infertility treatment within the framework of ART programs and increases their effectiveness.

According to WHO, infertility affects up to 15% of all pairs of reproductive age in the world and does not tend to decrease [1]. In the Republic of Kazakhstan, there is no reliable statistics on the frequency of infertile marriage. According to various data, the frequency varies from 12 to 15.5% [2 - 4].

According to the Order of the Ministry of Healthcare of the Republic of Kazakhstan No. 627, dated October 30, 2009 "On the Approval of the Rules for Conducting Assistive Reproductive Technologies" are methods of infertility therapy in which some or all stages of conception and early development of embryos are carried out outside the body and include: extracorporal fertilization and embryo transfer (ECO and ET) and gametes into the uterine cavity, intracytoplasmic sperm injection (ICSI), surrogate motherhood, preimplantation genetic diagnosis and artificial insemination with the sperm of the husband (donor), donor of oocytes.

The appointment of genetic research methods for couples with infertility is determined based on the etiological factor and the form of infertility and is not included in the list of compulsory volume of research.

According to the world literature, the ratio of the etiologic factors of infertility in human is: the female factor is 30%, the male factor is 30%, the mixed factor is 30% and idiopathic infertility, when the etiology is unknown - 10% [4,5]. Cases of idiopathic infertility are associated with insufficiently studied genetic factors regulating spermatogenesis and reproduction in general, idiopathic infertility in men accounts for up to 30% of cases, and when molecular genetic and cytogenetic diagnostic methods are used, the frequency of revealing reproductive disorders of the genetic nature increases significantly [5-8].

One of the most obvious current trends is the growth of the male factor of infertility in the Republic of Kazakhstan (49%), and in this connection, half of couples with infertility from the total number of resorts to ART clinics needs in ICSI (intraplasmic sperm injection) [3,6,7].

An important role in the genesis of reproductive disorders in men is played by various genetic factors - chromosomal abnormalities, as well as mutations of individual genesthat lead to deviations in the spermogram parameters: disturbances in the structure and quantity of spermatozoa that affect their mobility and fertility and are detected in 18.5 % of men suffering from severe forms of male infertility and require genetic diagnosis [5,7,9,10].

According to the results of a survey of spouses with reproductive problems, the frequency of chromosomal abnormalities varies from 5% to 18.4% [11-13]. Indications for a cytogenetic examination of this group of patients were abnormalities in the spermogram, a syndrome of premature ovarian failure, oogenesis abnormalities, unsuccessful IVF attempts, loss of pregnancy, the birth of a child with congenital malformations or chromosomal pathology [5,11,17,21]. The most frequent in karyotypes of patients with reproductive disorders are quantitative and structural anomalies of sex chromosomes and autosomes (translocations, inversions, marker chromosomes, deletions, duplications, mosaic variants of karyotypes with chromosomal abnormalities, pericentric inversion of the 9th chromosome), which do not manifest phenotypically, but may have a potential effect on sperm counts prior to oligoasthenospermia or azoospermia[12,13,17,20].

Particular attention should be paid to chromosomal polymorphism - structural chromosomal aberrations, which, according to the International nomenclature of chromosomes, are considered as paraphysiological variants of the normal karyotype and are the most frequent cytogenetic finding and occurs according to generalized data from 12.3% to 37.2% [12,13,17] . Chromosomal polymorphism includes: an increase or decrease in the heterochromatin regions of the Y-chromosome, an increase in satellites or satellite strands of acrocentric chromosomes.According to studies conducted in ART clinics in the Republic of Kazakhstan, chromosomal polymorphism was more common in men than in women (14.1% and 10%, respectively), an increase in heterochromatin sections of 1, 9, 16 autosomes and satellites/satellite strands of acrocentric chromosomes occurred in 10.2%, change in heterochromatin regions of the Y-chromosome in 4.7%, pericentric inversion of the 9th chromosome was 10.18% in women and 6.5% in men [35].

Many authors confirm the presence of adverse effects of chromosomal polymorphism on spermatogenesis, inefficiency of ART programs, miscarriage, congenital malformations and chromosomal pathology in offspring [13,17,18,19,35].

In addition to chromosomal abnormalities, the most common genetic causes of infertility in men are mutations of genes specifically involved in spermatogenesis. It is possible to distinguish the most significant: deletions microdeletions of the long arm of the Y chromosome, absorbing AZF locus [5, 7, 10, 14] and mutations in the CFTR gene encoding the special transmembrane regulatory cystic fibrosis protein [5,11].

The microdeletion of the Y chromosome is the deletion (prolapse) of certain segments of the Y chromosome - the AZF locus (azoospermia factor). The AZF locus is located in the long arm of the Y chromosome (Yq11). Genes located in this locus play an important role in the process of spermatogenesis.

The deletions of the AZF locus of the Y chromosome represent the second most common genetic cause of disorders in spermatogenesis in men with infertility after chromosomal pathology and are associated with varying degrees of spermatogenesis disturbance from a moderate decrease in its activity (hypospermatogenesis) or a spermatogenesis block to a virtually complete absence of gametes in seminal tubules, the so-called Sertoli-Cellular Syndrome (SCS). The occurrence of deletions of the AZF-locus of the Y chromosome, according to different literature sources, is from 7 to 55% of men with pathozoospermia [9,14,15,16,20], in the RK - 11.2% [8].

There are three main AZF loci: AZFa, AZFb and AZFc. Deletions of AZFa genes cause: Sertoli-only cell syndrome, which is characterized by a complete absence of male germ cells, azoospermia, spermatogenic activity is less observed The share of microdeletions of the AZFa subregion accounts for only 5% of all Y-chromosome microdeletions, AZFb sub-region accounts for up to 16% of all microdeletions, the microdeletions of the AZFc subregion lead to 12% of non-obstructive azoospermia and 6% of severe oligozoospermia. According to many authors, approximately 65-70% of cases of Y-chromosome microdeletions occur in this area [8,15,16].

Studies by different authors show that men, carriers of the deletions of the AZF-locus of the Y chromosome, can receive their offspring in the ART program using high-tech methods of sperm selection

for fertilization of oocytes (ICSI/PICSI) with a comparable fertility rate and the onset of pregnancy similar to men, non-carriers of the deletions [7,9,11,15,16]. The determination of the dependence of the extent of spermatogenesis impairment on the size and location of deletions/microdeletions may have some prognostic value in terms of the possibility of obtaining spermatozoa suitable for carrying out ART programs. Thus, the presence of deletions that capture the AZFa and/or AZFb subregions, indicates that it is impossible to obtain mature germ cells, and in patients with AZFc deletions (with testicular biopsy, TESA/TESE or other methods), in about 50-70% of cases mature sperm can be obtained [11,15,16,20].

However, it should be noted that they have a risk of transmitting a given Y chromosome deletion to their sons (in 100% of cases), and an increased risk of chromosomal pathology in embryos that are viable and can have a high potential for implantation and further development, which can lead to birth children with genetic pathology (59.7%) [15,16,34].

Another equally important genetic cause of male infertility is the carriage of mutations in the CFTR gene - Cystic Fibrosis Transmembrane conductance Regulator. The CFTR gene is located on the long arm of the 7th chromosome.). This protein participates in the transport of chloride ions through the cell membrane and regulates the viscosity of secretion allocated by excretory glands and secretory epithelium cells of the respiratory system, digestion (pancreas, liver, biliary tracts, digestive tract), perspiratory glands and urogenital tract (epididymis head and deferent duct).

According to sumarized data, there is a positive correlation between the carriage of mutations in the CFTR gene and the congenital bilateral absence of the deferent duct (CBADD), more rarely, the unilateral (CUADD) - form of male infertility, which is a frequent cause of obstructive azoospermia. More than 95% of men with CBADD are carriers of mutations in the CFTR gene and up to 50% of men with idiopathic obstruction of the deferent ducts[20-22].

The spectrum of mutations in the CFTR gene in men with infertility is significantly different from patients with cystic fibrosis - they are not characterized by the presence of two mutations (homozygosity), they have mutations in the heterozygous state when one allele is represented by a mutant one. These male carriers have such changes in spermogram as oligoasthenoteratozoospermia, isolated oligozoospermia, azoospermia of unknown origin, reduced volume of seminal plasma, absence or low concentration of fructose, pathological viscosity of ejaculate [20-22]. Molecular genetic research - the search for mutations in the CFTR gene is one of the most important genetic tests in cases of male infertility with obstructive azoospermia.

Currently, six allelic variants of IVS8-T polymorphism have been described: frequently occurring 5T, 7T and 9T, a much less common 6T allele and extremely rare 2T and 3T alleles [22]. Recent studies show a higher incidence of 5T alleles in men with infertility in different populations - 21% [20-24]. Carrier frequency for individual mutations of the CFTR gene in men with infertility is 12% [5].

For men with CBADD/CUADD, the 5T allele is highly specific, which is found in 40-50% of patients, and with no clinical manifestations of cystic fibrosis [22]. However, in male carriers of mutations in the CFTR gene, there is a risk of having a child with cystic fibrosis, so when a mutation is detected, it is necessary to carry out a molecular genetic study of the partner.

Thus, before the introduction of couples in ART programs, it is necessary to conduct medical genetic counseling with the mandatory appointment of a cytogenetic and molecular genetic examination for men with pathospermia, and when detecting chromosomal pathology, AZF microdeletion in the Y chromosome and mutations in the CFTR gene, by mandatory informing them of risk of inheritance of genetic pathology by their offspring. Modern possibilities of high-tech methods of sperm selection for oocyte fertilization (ICSI/PICSI) and preimplantation genetic diagnosis allow for today to recommend effective ART programs in this category of patients, which allow to avoid empirical and often expensive forms of infertility treatment in men, but also to prevent the transfer of genetic pathology to offspring [32].

One of the relatively new molecular-cytogenetic methods of preimplantation diagnosis is comparative genomic hybridization on microchips (aCGH) in the detection of embryos with chromosomal pathology and is widely used in many ART clinics. A large number of studies have confirmed the high accuracy, sensitivity and reproductibility of this method. aCGH allows to analyze each of the 24 chromosomes and determine the increase or decrease in the abundance of chromosomal loci on a genome-wide scale [24-27], i.e. allows to analyze the chromosome set of the embryo. Selection of embryos with a normal set of

chromosomes increases the likelihood of a successful pregnancy and the birth of a live and healthy child up to 65% [33].

Chromosomal abnormalities, such as aneuploidy, unbalanced translocations, deletions and microdeletions, duplications and microduplications, are easily detected using the aCGH method. The limitation of the method is that polyploidy and balanced translocations or inversions cannot be detected, but, in the opinion of many authors, this is only a small restriction that does not affect its successful application in the clinical practice of ART [28,29]. To successfully overcome the limitations of this method, an individual approach to infertility treatment by ART methods is necessary, and the results should be evaluated together with cytogenetic methods [29].

The main advantage of the method of preimplantation genetic screening is the detection of microdeletions and microduplications that lead to the degeneration of embryos or the generation of offspring with congenital malformations and mental retardation causing a disability [30,31].

Numerous literature data testify to the need for medical genetic counseling of infertile couples with mandatory cytogenetic research, molecular genetic studies of men with pathospermia, and preimplantation genetic screening of embryos due to a high risk of transmission of genetic disorders to offspring, which significantly changes the tactics of treating infertility in ART programs and increases their effectiveness and avoids empirical and often expensive forms of infertility treatment in men, as well as to prevent the transfer of genetic pathology to the offspring, which determines the need to improve the algorithms of medical and genetic examination of couples with genetic pathology of the reproductive function to optimize the tactics of infertility treatment within the ART programs in the Republic of Kazakhstan.

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# ПЕРСОНИФИЦИРОВАННЫЙ ПОДХОД ПРИ ГЕНЕТИЧЕСКОМ СКРИНИНГЕ СУПРУЖЕСКИХ ПАР В ПРОГРАММАХ ВРТ (Обзор литературы)

**Аннотация.** Бесплодие – неспособность человека детородного возраста к воспроизводству потомства и представляет серьезную медико-социальную проблему. В последние годы для лечения бесплодия все чаще применяются вспомогательные репродуктивные методы технологии (далее - ВРТ). Однако, не смотря на современные достижения в лечении бесплодия методами ВРТ, проблема рождения здорового потомства остается не менее актуальной. Все шире при проведении ВРТ используются методы генетического скрининга для выбора генетически здорового эмбриона.

Диагностика генетических причин бесплодия у супружеских пар значительно повышает результативность ВРТ: увеличивает частоту имплантации и наступления беременности, снижает вероятность прерывания беременности и риск рождения потомства с хромосомной патологией и врожденными пороками развития.

*Методы исследования:*Проведенанализ современных данных литературы, подтверждающие важное значение генетических исследований в диагностике и лечении бесплодия.

*Результаты:* В представленном обзоре литературы, продемонстрирована необходимость проведения медико-генетического консультирования супружеских пар с бесплодием с обязательным цитогенетическим исследованием обоих супругов,молекулярно-генетическим исследованием мужчин с патоспермией и обязательным проведением преимплантационной диагностики полученных эмбрионов в связи с высоким риском передачи хромосомной патологии потомству, что значительно меняет современную тактику лечения бесплодия в рамках программ ВРТ и повышает их эффективность.

**Ключевые слова:** хромосомная патология, хромосомный полиморфизм, делеции AZF-локуса Y-хромосомы, мутации в гене CFTR, преимплантационный генетический скрининг методом aCGH.

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