

A New Perspective On Preimplantation Genetic Diagnosis In The Republic Of Uzbekistan (Literature Review)

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Abstract: Preimplantation genetic diagnosis has existed for more than 20 years, during which time the range of possibilities of assisted reproductive technologies and the potential of molecular genetic diagnosis of single cells have significantly expanded. To date, preimplantation diagnosis has evolved from an experimental procedure to a valid and earliest form of prenatal diagnosis, while expanding the range of indications. Women of older reproductive age - 35 years and older. Couples with a history of more than three spontaneous early terminations of pregnancy (habitual miscarriage). Men with severe disorders of spermatogenesis - oligoasthenoteratozoospermia, severe oligozoospermia, azoospermia. Couples with repeated unsuccessful IVF attempts - more than three unsuccessful attempts. Mitochondrial diseases caused by nuclear DNA mutations are transmitted according to Mendelian laws, hence the pattern of PGD should be the same as for monogenic diseases. The inheritance of mitochondrial diseases caused by mtDNA mutations is complicated by heteroplasmy (genetic heterogeneity of the mitochondrial population), different levels of heteroplasmy in different oocytes, random distribution of mtDNA molecules between blastocyst cells, and the fact that the proportion of mutant mtDNA molecules may vary differently in tissues during the development and life of the organism. This has transformed PGD from an experimental procedure to a valid and earliest form of PD, while expanding the range of indications.

Keywords: Preimplantation genetic diagnosis; prenatal diagnosis.

Introduction: Prevention of the birth of children with hereditary diseases is one of the urgent tasks of modern medicine. Currently, to accomplish this task, methods of prenatal diagnosis (PD) are used, aimed at identifying pathological conditions in the fetus during progressive pregnancy, and preimplantation genetic diagnosis (PGD / PGD - Preimplantation Genetic Diagnosis), which allows to study the genome of the embryo before its transfer to the uterine cavity. PD of monogenic and chromosomal diseases is performed mainly using invasive methods for obtaining fetal material (chorionic or placental villus biopsy, amniocentesis, cordocentesis) and subsequent molecular genetic or cytogenetic studies. If PD reveals a genetic disorder in the fetus, then the pregnancy can

be interrupted artificially. In some cases, spouses who are aware of the identified pathology in the fetus decide to continue the pregnancy. The adoption of such a decision must be necessarily consulted by a specialist; a married couple must understand the responsibility that falls on them and be aware of the need for large material and moral costs associated with the care and social adaptation of such children. It is important that spouses know that the life expectancy of people with a genetic pathology is much less than in the normal population. PGD, unlike PD, is performed before implantation of the embryo into the uterine wall. Carrying out genetic diagnostics before implantation is possible only in case of in vitro fertilization, that is, when using Assisted Reproductive Technologies (ART).

The PGD procedure involves biopsy of one or more cells from an oocyte (polar bodies) or embryo (blastomeres, trophectoderm cells) and their subsequent genetic testing for gene or chromosome mutations. In the absence of test pathology, the embryo can be transferred into the uterine cavity or cryopreserved until transfer in the next IVF cycle (Fig. 1). Now the most promising is the diagnosis of embryos at the blastocyst stage, with the study of 3–8 cells of the trophectoderm.

The study aimed to: To date, preimplantation diagnosis has evolved from an experimental procedure to a valid and earliest form of prenatal diagnosis, while expanding the range of indications.

METHODS

Currently, for the genetic study of embryonic cells, fluorescent in situ hybridization (FISH - Fluorescence in situ hybridization) or polymerase chain reaction (PCR / PCR - Polymerase chain reaction) is usually used. Less commonly used is a more expensive method - comparative genomic hybridization using microarrays (array comparative genomic hybridization – a CGH). Modern possibilities of preimplantation genetic diagnosis are developing rapidly, and, apparently, will expand the possibilities of IVF with PGD in the near future. Indications for PGD are divided into several groups (Table 1).

Table 1 – Diagnostic methods in at-risk patients

Indication	Patient group	Diagnostic methods
High genetic risk of transmission of hereditary pathology to offspring	carriers of gene mutations that cause monogenic diseases: autosomal recessive, autosomal dominant, linked to the X or Y chromosome)	PCR
	-carriers of chromosomal abnormalities: numerical and structural aberrations of chromosomes	FISH, PCR, a-CGH, SNParrays
High risk of formation of aneuploid gametes with normal somatic karyotype	<ul style="list-style-type: none"> • women of older reproductive age — 35 (38) years and older; • couples with a history of more than three spontaneous abortions in early pregnancy (recurrent miscarriage); • men with severe disorders of spermatogenesis: oligoasthenoteratozoospermia, 	FISH, PCR, a-CGH, SNParrays

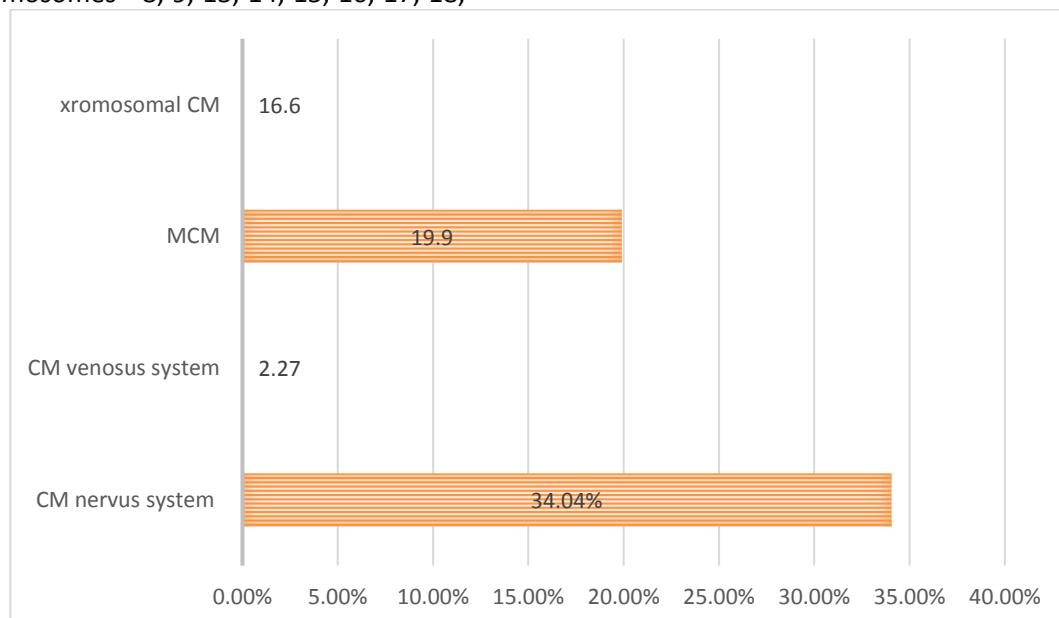
	severe oligozoospermia, azoospermia; • couples with repeated IVF failures (more than three failures of unknown cause)	
A high risk of developing a severe disease in the offspring with late manifestation, incl. oncological disease	carriers of gene mutations that significantly increase the risk of developing cancer and diseases with late PCR manifestation	PCR
The presence in the family of a child with a hematological disease who needs a transplant of donor hematopoietic stem cells to continue life	carriers of hereditary hematological diseases; • families with a child with a sporadic hematological disease	PCR
High risk of severe mitochondrial disease in offspring	female carrier of a specific mtDNA mutation	PCR
Family planning	married couples who, for objective reasons, plan to have a child of a certain gender	FISH, PCR

The first group is a high genetic risk of transmission of hereditary pathology to offspring. Such diagnostics are carried out for carriers of gene and chromosomal abnormalities. These can be monogenic diseases (autosomal recessive, autosomal dominant, linked to the X or Y chromosome), as well as chromosomal

abnormalities (numerical and structural aberrations of chromosomes). It is important for a geneticist whose task is to help a family have a healthy child, it is important to remember that among patients in need of such a diagnosis, there may be patients with gonadal mosaicism. This means that a genetic analysis of a

patient's blood sample will not show the presence of a mutation. It is possible to assume gonadal mosaicism in a patient according to the anamnestic analysis of his family - a study of sick children in the family, abortion material from previous pregnancies and embryos obtained in IVF cycles. The second group is the presence of a high risk of formation of aneuploid gametes with a normal somatic karyotype. In this case, the number of certain chromosomes in pre-implantation embryos is analyzed. This diagnosis is called Preimplantation Genetic Screening (PGS - Preimplantation Genetic Screening). Currently, FISH can routinely determine the number of 9–12 chromosomes. The chromosomes to be tested are selected based on their significance for prenatal and postnatal development. It is advisable to test the number of chromosomes - 8, 9, 13, 14, 15, 16, 17, 18,

21, 22, X, Y [8, 12]. It has been shown that determining the number of five chromosomes makes it possible to identify 40%, and twelve chromosomes about 90% of all chromosomal abnormalities in embryos that have reached the blastocyst stage [18]. The a CGH method allows you to determine the number of all human chromosomes and investigate the imbalance in individual chromosome segments. In the last three years, European countries and the United States have begun to actively use the a CGH method for PGD [2,8,9]. The use of SNP-arrays, microarrays based on single nucleotide substitutions (SNP, single nucleotide polymorphism), makes it possible to simultaneously label more than a million chromosomal loci [5,8].



Picture 1. Results of screening analyzes of the incidence

RESULTS

The prospects for a CGH for PGS are obvious, but so far, along with a high cost, the method has disadvantages: the inability to determine the ploidy of a cell and the inability to distinguish a karyotype with a balanced structural rearrangement of chromosomes from a normal one. The following groups of patients have an increased risk of formation of aneuploid gametes: 1. Women of older reproductive age - 35 years and older. 2. Couples with a history of more than three spontaneous abortions in the early stages (recurrent miscarriage). 3. Men with severe disorders of spermatogenesis - oligoasthenoteratozoospermia, severe oligozoospermia, azoospermia. 4. Married couples with repeated IVF failures - more than three unsuccessful attempts (it should be remembered that this group is especially heterogeneous, other factors of

IVF failure must be excluded). It is important to remember that in all patients referred for PGS, a normal karyotype must be confirmed, and for patients in groups 2, 3, and 4, non-genetic causes of the pathology must be excluded. PGS accounts for about 60% of all PGDs performed in the world, however, the importance of pre-implantation screening for aneuploidies has been debated among IVF specialists for five years. PGS began to be carried out in the early 1990s [12, 14] in order to improve the results of IVF by transferring embryos without aneuploidy into the uterine cavity, the most significant for pre- and postnatal development. Since numerous studies have shown that 50–60% of spontaneous abortions of early pregnancies are associated with chromosomal pathology in the fetus [9, 20], it was clear that when embryos are transferred after PGS, the frequency of spontaneous pregnancy losses will be less and,

consequently, the IVF result better. In addition, when transferring embryos without frequent chromosomal abnormalities, an increase in the frequency of implantation was expected. By 2010, numerous results were obtained indicating that PGS increases the frequency of implantation, reduces spontaneous early pregnancy losses, and increases the frequency of birth of healthy children ("taking home baby") after IVF in families at risk [3,4,8]. Most of the published data on PGS come from non-randomized retrospective studies with a high level of significance (2nd or 3rd level of significance), however, randomized trials (RI) in this area are almost non-existent. In 2023, a Dutch group of specialists published the results of a large RI of the effectiveness of OPO [11]. The results indicated a negative effect of PGS on the outcomes of IVF cycles, which was the beginning of an ongoing discussion among various specialists in reproductive medicine. The study of the Dutch group has many significant methodological shortcomings, which are detailed in a number of authoritative publications [6, 8]. These shortcomings make it untenable to draw conclusions about the significance of PGD in IVF, but it cannot be said that this study was very useful in revising the indications for PGS, methodological progress in PGD, developing a quality control system in PGD, and even stimulating research in the field of human embryology. To date, only 12 RIs on PGS have been published [1,2,4,8,9,10], and a meta-analysis of these studies has been carried out. All published RIs have significant shortcomings (controversial methodological approaches, insufficient number of patients, organization - "design" of the study, etc.), which makes it impossible even with the help of a meta-analysis to draw an unambiguous conclusion about the effect of PGS on the IVF outcome. It is clear that an incorrectly organized PGS will not improve the outcome of IVF, and further RI in the form in which it was carried out does not make sense and is unethical in relation to patients. All experts agree that a high level of mosaicism in preimplantation human embryos reduces the positive effect of preimplantation screening for aneuploidies—new methodological approaches are needed, as well as additional studies of the significance of mosaicism for different stages of human embryonic and prenatal development. Despite the controversy around PGS, it is clear that, if properly organized, the method significantly expands the possibilities of IVF and can be called the earliest PD method that prevents the implantation of embryos with complete and partial aneuploidies and, therefore, prevents spontaneous abortions and the birth of children with chromosomal pathology. The third group - carriage of gene mutations that can cause diseases with late manifestation, manifested in adulthood or old age, including

mutations predisposing to the development of oncological diseases. Such carriage was not a standard indication for PD, however, in some cases, for example, with a mutation in the RB1 gene predisposing to the occurrence of retinoblastoma, the probability of developing the disease in offspring in the case of transmission of the mutant allele is about 90%. Doing PD with subsequent possible termination of pregnancy for this group of patients is not considered justified. PGD, in contrast, may be chosen by spouses to prevent transmission of the disease to the next generation. Currently, PGD regimens have been developed for oncological diseases such as Li-Fraumeni syndrome (p53), retinoblastoma (RB1), familial polyposis (APC), neurofibromatosis I (NF1) and II (NF2), familial breast and ovarian cancer (BRCA1), BRCA2, ataxiatelangiectasia (ATM), etc.. The results of successful PGD in early-onset familial Alzheimer's disease (APP gene mutation) have been published. The fourth group is the presence in the family of a child with a hematological disease who needs a transplantation of donor hematopoietic stem cells to continue life. For the first time, such PGD was performed 11 years ago to save a girl with Fanconi anemia. After that, for several years, European experts discussed the ethics of such PGD and came to the conclusion that this diagnosis has a right to exist. Currently, the treatment of sick siblings with bone marrow transplantation of healthy HLA-identical siblings born as a result of IVF with PGD is not a rare event. It is clear that PD is impossible in this case, the theoretical probability ISSN 1684–0461 sick child is low. To date, there are the following indications for PGD with HLA-typing: hereditary diseases complicated by bone marrow insufficiency, hemoblastoses, immunodeficiencies, and metabolic diseases such as X-linked adrenoleukodystrophy. In addition, preimplantation HLA typing is used to treat sporadic hematological diseases; for the first time, the method was used to treat a sibling patient with hypoplastic Blackfan–Diamond anemia. The fifth group is the carriage of genetic diseases caused by mutations in mitochondrial DNA (mtDNA). Since the zygote receives all the mitochondria from the egg, these diseases are maternally inherited. Mitochondrial diseases caused by nuclear DNA mutations are transmitted according to Mendelian laws, therefore, the PGD regimen should be the same as for monogenic diseases. The inheritance of mitochondrial diseases caused by mtDNA mutations is complicated by heteroplasmy (genetic heterogeneity of the mitochondrial population), different levels of heteroplasmy in different oocytes, random distribution of mtDNA molecules between blastocyst cells, and the fact that the proportion of mutant mtDNA molecules can change differently in tissues during development and life of the organism. The manifestation of diseases

caused by mtDNA mutations is very variable, the symptoms of some diseases vary both between families and within the same family. Even though disease severity depends on a particular mtDNA mutation and the number of mutated molecules, there are numerous exceptions. The difficulty of predicting the course of many mitochondrial diseases, and the impossibility of selecting embryos completely free of mutant mtDNA during PGD, makes it very difficult to perform this kind of PGD [1]. Currently, it is recommended to develop PGD for mtDNA mutations within the research protocol and to inform potential parents that the first cycle of PGD can only be performed to collect information regarding the reliability of the method [3]. To date, few data on clinical cycles of PGD in mtDNA mutations have been published [4]. The sixth group is the determination of the sex of embryos for the purpose of family planning. In European countries and the United States, such a diagnosis is called "social sexing". According to the PGD consortium ESHRE (European Society of Human Reproduction and Embryology), there is a decrease in such diagnoses worldwide. According to the latest published data of the consortium, the share of such PGD in 2007 was 1.6%, and in the previous nine years it was 2.7% [10]. Carrying out PGD for the purpose of family planning cannot be considered unambiguously justified. However, if we compare this method with PD carried out for the purpose of family planning, where in most cases, when a fetus of an "unwanted" sex is identified, an artificial termination of pregnancy is assumed, PGD seems to be more humane. Since November 2011, sex selection of pre-implantation embryos for transfer without medical indications has been prohibited by law in the Russian Federation [1]. Despite the fact that pre-implantation diagnostics has been carried out since 1989, PGD methods and coordination of processes during diagnostics have not yet been standardized. In recent years, worldwide attention has been paid to standardization, internal and external quality control of PGD procedures. This is very important to reduce the error of the method, which, if the relevant requirements are not met, can be significant. Standardization of PGD procedures improves the quality of services, creates optimal conditions for the work of specialists, and increases the security of patients. This is especially true when the diagnosis is carried out according to the "transport scheme" - that is, different stages of IVF with PGD are performed in different medical institutions located in different cities or even countries. The "transport scheme" is widely used in the Americas and Europe. This is due to the fact that it is not yet economically viable to maintain expensive equipment for genetic research and a staff of highly qualified employees in

small and medium-sized ART clinics. Clinical and laboratory departments are connected using special couriers and standard means of communication (Internet, telephone). The outcome of IVF cycles with PGD largely depends on the clarity of coordination between departments. Currently, two large international medical organizations: the PGD Consortium (ESHRE) and the International PGD Community (PGDIS) continue to develop standards for PGD procedures American specialists — American Society for Assisted Reproductive Technology (SART) with American ISSN 1684-0461 Reproductive Medicine (ASRM - American Society for Reproductive Medicine) also work in this area.

CONCLUSION

Recently, projects on external quality control of PGD procedures have been launched in Europe: by the FISH method [11] and by the PCR method. International PGD communities, individual authorities in the field of PGD and regulatory medical authorities in different countries recommend that all PGD laboratories be accredited in accordance with internationally recognized quality standards or conduct activities that will facilitate such accreditation in the future. To date, two programs of the International Organization for Standardization - ISO (ISO - International Organization for Standardization) have been tested in Europe. Here we present a possible scheme for carrying out PGD procedures, taking into account the interaction between different departments of the ART clinic and the genetic laboratory, taking into account PGD according to the "transport scheme" (Fig. 2). This flowchart is designed to standardize the processes involved in PGD, to easily identify the different stages and checkpoints of a procedure, and to establish links between them. The scheme is necessary for quick quality control of the work of specialists (doctors of reproductologists, geneticists, genetics laboratory assistants, biologists, paramedical personnel, etc.) and convenient coordination of their actions. PGD has been developing for more than 20 years, during this time there has been significant progress in IVF techniques, new methods and equipment have appeared that allow expanding the range of ART capabilities. The changes that have taken place during this time in molecular diagnostics are even more impressive; at present, PGD has in its arsenal accurate and universal methods that make it possible to study many genetic markers of a single cell in less than a day. As a result, PGD has evolved from an experimental procedure to a valid and earliest form of PD, while expanding the range of indications. Further development of molecular diagnostic methods, their standardization for PGD, combined with improved organization and

coordination of processes in IVF cycles with PGD, promises good prospects.

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