GENETIC FACTORS AND GENETIC METHODS FOR STUDYING INFERTILITY IN MEN

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ГЕНЕТИЧЕСКИЕ ФАКТОРЫ И ГЕНЕТИЧЕСКИЕ МЕТОДЫ ИССЛЕДОВАНИЯ БЕСПЛОДИЯ У МУЖЧИН

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Annotation. Infertility is a social problem. It is believed that if, with regular sexual activity without contraception, pregnancy does not occur within a year, it is necessary to begin examination and, possibly, treatment of the spouses. The article presents data on the genetic factors of male infertility and discusses methods that can be used to diagnose it. An in-depth study of the sperm of infertile men at several levels of organization of genetic material will make it possible to evaluate the information content of each method separately and in combination, as well as to develop an optimal

algorithm for diagnostics in order to select the most effective method of treating male infertility.

Key words: male infertility, types of infertility, genetic factors, genetic methods.

Аннотация. Бесплодие является социальной проблемой. Считается, что если при регулярной половой жизни без контрацепции беременность не наступает в течение года, необходимо начинать обследование и, возможно, лечение супругов. В статье представлены данные о генетических факторах мужского бесплодия и рассмотрены методы, которые могут быть использованы для его диагностики. Углубленное изучение спермы бесплодных мужчин на нескольких уровнях организации генетического материала позволит оценить информативность каждого метода отдельно и в комплексе, а также разработать оптимальный алгоритм для проведения диагностики с целью выбора наиболее эффективного метода лечения мужского бесплодия.

Ключевые слова: мужское бесплодие, виды бесплодия, генетические факторы, генетические методы.

Introduction. Infertility is primary if there has never been a pregnancy, and secondary if a woman has had at least one pregnancy, no matter what the outcome (childbirth, ectopic pregnancy, miscarriage, etc.). In female infertility, there are several forms: tubal, peritoneal, tubal-peritoneal, endocrine, endometriosis-related infertility, immunological, psychological, etc. The combination of several causes of infertility in a woman is called “combined infertility.” Combined infertility must be distinguished from combined infertility, in which both a man and a woman have problems with reproductive health. In addition to these forms, there is also such a form as idiopathic infertility, or infertility of unknown origin, which is observed among absolutely healthy and well-compatible married couples.

When a woman cannot get pregnant for a certain time, she seeks advice from a doctor. If it turns out that she is completely healthy and can have a child, then the obstetrician-gynecologist recommends that her spouse be examined. However, not every man is ready to calmly admit that he needs medical advice and help.

Today it has been established that the causes of male infertility, as well as female infertility, are very diverse. Among the causes of male infertility are ejaculatory, sexual, anatomical changes in the structure of the genital organs, endocrine disorders, inflammatory processes, immunological factors, various disorders of spermatogenesis, environmental factors and much more. Today, among the causes of infertile marriage, male infertility accounts for up to 40%, and it should be given the same close attention as female infertility [3, 9].

It is known that genetic factors account for at least 30-50% of all cases of severe forms of infertility in men. Spermatogenesis is a complex biological process that

depends on a precisely controlled cascade of activation and deactivation of specific genes. The result of the work of these genes is the process of maturation of sperm from precursor cells. In humans, more than 2000 genes are involved in this process. Due to genetic disorders, forms of infertility that differ in their etiology and severity can occur: from minor disturbances of spermatogenesis to complete dysfunction of the gonads.

Among the genetic factors of male infertility, there are three main ones: changes in the genetic apparatus at the chromosome level (chromosomal aberrations), at the level of a gene or group of genes (mutations), at the level of total DNA (chromatin dispersion and DNA fragmentation). That is why, in addition to standard morphological and biochemical tests for male infertility, it is recommended to use molecular cytogenetic and molecular genetic methods that allow one to assess the state of the genetic apparatus of somatic and germ cells in men.

Cytogenetic analysis, or karyotyping, allows you to see changes at the chromosome level in somatic cells (for example, blood lymphocytes) and determine the constitutional characteristics of the patient.

The external genitalia in such patients, as a rule, are formed according to the male type; they are characterized by microorchidism, which is one of the most important clinical criteria for these syndromes. The ejaculate volume rarely reaches 1.5 ml, and azoospermia manifests itself. When oligozoospermia is detected, it is advisable to conduct a molecular cytogenetic (FISH test) study of ejaculate cells to identify the mosaic form of the syndromes. With a mosaic form with a predominance of the 46,XY clone, fertile men have been described, although they, as a rule, have an increased frequency of specific and nonspecific chromosomal aberrations (breakages) in sperm [3, 17]. When a patient is diagnosed with azoospermia, but with the presence of sperm precursor cells in the testicles, it is possible to use artificial insemination methods with the collection of genetic material directly from the testicle by biopsy. The birth of healthy children conceived in this way has been described. At the moment, it is also possible to use the method of preimplantation genetic diagnosis to select embryos with a normal set of chromosomes before embryotransfer in an in vitro fertilization (IVF) cycle.

The frequency of structural chromosomal aberrations in the karyotypes of men with infertility varies in different studied samples and ranges from 1.6–4.2% [2, 13]. According to other authors [1, 5], in the group of patients who are candidates for in vitro fertilization using the ICSI method (intracytoplasmic sperm injection, ICSI), this figure reaches 13.1%. In the latter case, the indications for cytogenetic examination were male factor infertility and unsuccessful IVF attempts.

In men with infertility, karyotypes with chromosomal aberrations such as balanced rearrangements are found: translocations (Robertsonian and reciprocal), marker chromosomes, inversions. According to the literature, if the population

frequency, for example, of such balanced translocations does not exceed 0.1%, then their frequency in groups of men and women with reproductive problems reaches 3.0-6.2 and 0.7-9.8%, respectively [2, 15]. Among the balanced rearrangements in the karyotypes of men, the most often detected is a derivative chromosome, which was formed as a result of a translocation between the 13th and 14th chromosomes - der(13;14) (q10;q10) [6, 7]. It is important to know that balanced rearrangements of chromosomes during their formation do not lead to the loss or addition of genetic material, but only to its movement within the genome. Their carriers, as a rule, are phenotypically normal and healthy, but have a risk of having a child with a chromosomal abnormality. On the contrary, the presence of unbalanced rearrangements (deletions and duplications) in the patient's karyotype changes the dosage ratio of genes, so their carriage is associated with significant deviations from the norm.

FISH (fluorescence in situ hybridization) is a molecular cytogenetics technique that can accurately identify a specific chromosome or parts thereof. This is especially important when diagnosing chromosomal microanomalies: microdeletions/microduplications (comparative genomic hybridization, or CGH), microdeletion syndromes not detected by traditional karyotyping, chromosome mosaicism, and much more. The FISH method is widely used to study the interphase nuclei of germ cells (spermatozoa) for the detection of numerical chromosomal abnormalities (aneuploidy). Such anomalies can occur in men with both a normal karyotype and an altered one, but with different frequencies. It has been proven that in the latter group their frequency is higher [1, 8].

Molecular genetic methods must be used to exclude mutations at the level of a gene or group of genes (AZF locus, CFTR gene mutations, determination of the number of CAG repeats in the AR gene associated with changes in sensitivity to androgens, and many others).

In addition to karyotype abnormalities, the most common genetic cause of infertility in men is Y-chromosome deletions involving the AZF (Azoospermia factor region) locus. Deletions of the AZF locus are associated with varying degrees of impairment of spermatogenesis - from a moderate decrease in its activity (hypospermatogenesis) to the almost complete absence of germ cells in the seminiferous tubules - "Sertoli cells only" syndrome.

It is customary to recommend karyotype determination and analysis of Y-chromosome microdeletions for all infertile men with a sperm count in the ejaculate of less than 5 million/ml, as well as for men from married couples who are planning an IVF or ICSI program. Based on the results of genetic tests and medical genetic counseling, the degree of risk of having children with reproductive dysfunction is assessed. Also, to assess the nature of the origin of Y-microdeletions (de novo mutation

or inherited), a molecular genetic examination of the father, brothers and other men of the proband's family is necessary.

Boys born after the use of ICSI to fathers with microdeletions in the Y chromosome are subject to clinical observation to assess their fertile status.

CFTR gene mutations. Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a protein involved in the transport of chlorine ions across the cell membrane. The gene encoding this protein has the same name. The presence of mutations in both copies of the CFTR gene leads, as a rule, to the development of the most common hereditary autosomal recessive monogenic disease - cystic fibrosis, and can also be a cause of male infertility [12]. The human CFTR gene is located on the long arm of chromosome 7 in the q31 region. Currently, more than 900 types of different mutations of the CFTR gene are known. About 70% of cases of cystic fibrosis are caused by the deletion of three base pairs encoding the amino acid phenylalanine at position 508 of the transmembrane regulatory protein - delF508(?F508). In addition, obstructive azoospermia observed in men in 25% of cases is a consequence of unilateral or bilateral congenital absence of the vas deferens, which arose due to mutations in the CFTR gene. Therefore, screening before the ICSI procedure necessarily includes molecular genetic studies of this gene. To diagnose this mutation, the real-time PCR method is used.

In the genetic determination of male-type development, the formation of testicles, and spermatogenesis processes, the SRY (Sex-determining Region Y) gene, which is located in the short arm of the Y chromosome (Yp11.3), is especially important. It is in this gene that the largest number of mutations associated with gonadal dysgenesis and/or sex inversion have been found. In the absence of a chromosomal section containing the SRY gene, or a mutation in the specified gene, the phenotype will be female with a male karyotype of 46,XY (Swyer syndrome). On the contrary, with a female karyotype of 46,XX, but in the presence of a locus with the SRY gene inserted as a result of translocation into the X chromosome or even an autosome, the phenotype will be male (de la Chapelle syndrome). But such men are usually infertile. Testing for the presence of the SRY locus can be carried out using the FISH method, and mutations in this locus are detected by PCR methods in addition, of course, to traditional karyotyping.

The gene encoding the androgen receptor. Another determining factor in male infertility is a violation of the hormonal regulation of spermatogenesis, in which the male sex hormones androgens play a key role. They interact with specific androgen receptors, determining the development of male sexual characteristics and activating spermatogenesis. The androgen receptor gene is characterized by the presence of a CAG (cytosine-adenine-guanine) repeat sequence. The gene encoding the androgen receptor is located on the X chromosome. Androgen receptors are found in the cells of the testes, prostate, skin, cells of the nervous system and other tissues The sensitivity

of the receptor to testosterone depends on the number of repeats in the androgen receptor gene, and the relationship is inversely proportional: the more repeats, the less sensitive the receptor. With an increased number of CAG repeats in men, the risk of developing oligo- and azoospermia increases. The upper limit of normal for determining the risk of genetic predisposition to a hormone-dependent disorder of spermatogenesis is 23 CAG repeats. According to some sources, the range of 20-26 repeats is considered a relative norm [2, 12].

Thus, when planning a pregnancy, comprehensive genetic testing for chromosomal and major gene mutations helps to identify problems in the male line that are not detected by other tests (biochemical, cytological, immunological, etc.), and to decide on the patient's treatment tactics.

In recent years, more and more evidence has been accumulating that, in addition to chromosomal and gene mutations, changes in the structure of sperm DNA itself play a significant role in the problem of infertility. The hypothesis that a decrease in reproductive function is sometimes associated with a pathological state of the total DNA of sperm has gained wide popularity.

Since normally DNA must have a certain conformation, chemical and physical structure, any minor damage to DNA or its packaging can lead to incorrect development of events after the penetration of such a defective sperm into the egg [1, 7]. It is important that a defective sperm does not always look pathological in appearance. The connection between the state of sperm DNA and spermogram parameters has not yet been proven And this is especially important during the ICSI procedure, since sperm that are selected for a cycle based on normal morphology may have damage at the level of the DNA molecule. According to numerous literature data, a decrease in the number of sperm with damaged DNA significantly increases the chances of getting a pregnancy that develops normally [3, 16]. Conversely, sperm with fragmented DNA can affect the early stages of embryonic development, especially the formation of the blastocyst. Such a pregnancy freezes in the early stages of embryo development.

The pathophysiological mechanisms leading to DNA fragmentation are not entirely clear. It is assumed that they may be caused by unrepaired DNA damage, defects in chromatin remodeling that occur during spermatogenesis, oxidative processes and apoptosis (programmed cell death). Spermatozoa are extremely sensitive to apoptotic stimuli, such as high doses of chemotherapy, genotoxic environmental factors (for example, smoking), etc.

Conclusions. From the above it follows that the causes of male infertility often do not lie on the surface, but require careful study simultaneously at several levels. Only by comparing the data of morphological, biochemical, cytogenetic and molecular

studies can one judge the patient's real reproductive potential and choose the appropriate treatment tactics.

An in-depth study of the sperm of infertile men at several levels of organization of genetic material will make it possible to evaluate the information content of each method separately and in combination, as well as to develop an optimal algorithm for diagnosing male infertility. Perhaps an integrated approach, namely a comprehensive study of sperm quality, will provide a more complete picture of the pathological process and, therefore, greater effectiveness of treatment.

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