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Transmission of male infertility and intracytoplasmic sperm injection (mini-review)

Відомо, що генетичні чинники зумовлюють приблизно третину всіх видів чоловічої стерильності. Цей факт необхідно враховувати при використанні штучних репродуктивних технологій у випадках, коли донор хворий на азооспермію (відсутність сперматозоїдів у спермі) або тяжку олігоспермію (концентрація сперматозоїдів у спермі становить менше ніж 5 млн/мл). Спадкова стерильність може бути спричинена генними мутаціями, кількісними або структурними аномаліями статевих хромосом чи аутосом. Цей огляд присвячений аналізу можливостей спадкової передачі чоловічої стерильності при впровадженні штучних репродуктивних технологій. Найважливішими чинниками випадків тяжкої олігоспермії та азооспермії є синдром Кляйнфельтера, мутації гена cystic fibrosis transmembrane conductance regulator (CFTR), котрі спричиняють кістозний фіброз, і мутації в зоні фактора азооспермії (azoospermia factor zone – AZF) Y-хромосом. Вірогідність мутації гена CFTR при передачі до наступної генерації становить 50%. Імовірність спадкової передачі синдрома Кляйнфельтера при мозаїчному кариотипі може сягати 70 %. Імовірність передачі AZF мутації по чоловічій лінії внаслідок використання штучних репродуктивних технологій становить 100 %. Відсоток чоловіків із AZF-мутаціями, що звертаються до центрів штучної репродукції, варіює від 3,2 до 14 %. Таким чином, принаймні 3,2 % усіх хлопчиків, що з'явилися на світ за допомогою цих технологій, будуть стерильні завдяки мутації в зоні AZF Y-хромосоми. Слід відзначити, що генетичний аналіз і консультування далеко не завжди проводяться перед початком циклу штучного запліднення. Передбачувані батьки (Parents-to-be) не завжди мають можливість ознайомитись із ступенем генетичного ризику їхньої майбутньої дитини і прийняти виважене та відповідальне рішення. Проте є можливість вирішити не використовувати штучні репродуктивні технології, а компенсувати свою неплідність, здійснюючи, наприклад, усиновлення. Відповідальність за високий ризик народження дитини з генетичними аномаліями лежить не тільки на батьках, але й на суспільстві, включаючи відповідальність наукових дослідників, що працюють у цій сфері, лікарів і законотворців.

For past decades, the male is noted to be the more frequent cause of sterility in couples. New technologies including intracytoplasmic sperm injection (ICSI) have been proposed to treat male sterility. In most cases male sterility is connected with a considerable decrease (oligozoospermia) or total absence of sperm (azoospermia) in the ejaculate. Low levels of testosterone, blockage of spermatogenesis, retrograde ejaculation, and/or obstruction of ejaculatory ducts can lead to oligo- or azoospermia.

Microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESA) followed by ICSI (a procedure in which one sperm is injected by micromanipulation into a single egg) has led to possible paternity even for men with severe oligozoospermia or azoospermia. Nevertheless, this revolution in medical technology has not been accompanied by advanced knowledge of the etiology of sterility. This is one of the reasons why certain causes of male infertility remains unknown [7].

Genetic etiology is responsible for approximately 30% of all kinds of male sterility [20, 27]. This information had led to concerns regarding the application of ICSI. Congenital sterility could be caused by gene mutation, quantitative or structural abnormality of sexual

chromosomes or autosomes. The possibility of inherited transmission of male sterility within the context of applying artificial reproductive technologies will be analyzed in this review.

Chromosomal abnormalities

In the ordinary male population chromosomal anomalies occur with a frequency of 0.5 %. In patients with oligozoospermia this percentage is higher (5.6 %–6.8 %), and among patients with azoospermia this index increases up to 14 % [47]. Anomalies of sexual chromosomes are twice as frequent in comparison with autosomal anomalies [28].

Chromosomal defects can be classified as either numerical or structural. Structural anomalies include deletions, inversions, duplication of a portion of a chromosome, or translocation of part of a chromosome to another chromosome. Numerical anomalies can be classified as polyploid, which is the presence of cells containing multiple copies of all chromosomes or aneuploid which is the presence of cells with an addition or deletion of one or more chromosomes.

The most common numerical sex chromosome abnormality (aberration), which is seen in male infertility practice is the trisomy of X chromosome, or Klinefelter's syndrome.

Klinefelter's syndrome

Klinefelter's syndrome which was described for the first time in 1942, is one of the most common chromosome aberrations. It occurs in 1 of 500 men. Among men seeking care in infertility clinics, Klinefelter's syndrome occurs 30 times more frequently [12]. Klinefelter's syndrome is also the most common cause of sterility (4–6 %) [42].

The phenotype of men with Klinefelter's syndrome varies, but usually includes high stature, female hair distribution, small firm testes, decreased level of intelligence, diabetes mellitus, obesity, and an increased incidence of leukaemia and sterility. It was shown that the mortality of persons with this syndrome is 40 % more than that of healthy people. In the majority of cases, death is a consequence of infections, neurological diseases, or disturbances in circulation or respirations [5]. Patients with Klinefelter's syndrome always have a severe oligozoospermia or azoospermia. This group occupies 14 % of all cases of azoospermia [7].

Etiology of disease is based on nondisjunction of chromosomes during gametogenesis or, more rare, during mitosis in early embryonic age, which leads to creation of karyotype 47,XXY, XXXY or XXXXY. About half of all cases of Klinefelter's are caused by the wrong distribution of chromosomes in spermatogenesis [29].

15% of males with Klinefelter's's syndrome have a mosaic 46,XY/47,XXY karyotype with varying degrees of spermatogenic failure [13, 36].

Histological analysis of testes of patients with Klinefelter's syndrome usually reveals the atrophy of seminiferous tubules germinal cells, fibrosis of seminal ducts and hyperplasic cells of Leyding. Until recently only isolated instances of natural conception from men with Klinefelter's syndrome were described, which is why Klinefelter's has been associated with sterility. Phenotype of patients with mosaic karyotype is seen less frequently. Spermatogenesis is seen at different levels of intensity and it's very rare that these men can conceive naturally [23]. In the sperm of patients with mosaic karyotype there are spermatozoa, but 7.5 % of them carry aneuploidies of sex chromosomes, which is 19 times more than in healthy men (0.4 %) [13]. This phenomenon could be explained in two ways: 1) Spermatogonies with caritype 47, XXY complete meiosis and produce spermatozoa with two or more sex chromosomes, or 2) The anomalies of spermatogenesis are caused by hormonal disorders , which is part of the pathogenesis of this disease.

Application of ICSI in combination with MESA or TESA give men with Klinefelter's syndrome the hope of having children [41].

Westlander et al [52] and Madgar et al. [33] both document success in receiving spermatozoa in 21–24 % of these patients [6].

High amounts of sperm with aneuploidy of the sex chromosomes create a risk to descendants of getting the same defect. In a study of Bielanska et al [4], 70 % of embryos generated from patients with Klinefelter's syndrome had chromosomal aberrations. This high risk of chromosomal disorders makes the case for patents to attend to pre-implant or invasive prenatal diagnosis, which is very traumatic in se, with selective abortion being an option in the event of positive test results [36]. Elevated invasivity of pre-implant diagnosis and eventual selective choices also after post-implant diagnosis are the causes of loose of human embryos, which, as well, were obtained by so complicated way.

Applying artificial technologies in men with Klinefelter's syndrome non-mosaic karyotype, has a very low chance of leading to conception. For this reason, it is very difficult to determine the risk of inheriting this anomaly. Some authors suggest the risk is not high. For instance, Tachdjian et al. [42] represent the data of 32 children, conceived with ICSI from men with Klinefelter's syndrome of non-mosaic karyotype. Among the 32 children only one (3 %) carried the syndrome. Nevertheless, Staessen et al [40] present results that are more troubling. Among embryos conceived by fathers with Klinefelter's syndrome of non-mosaic karyotype 13.2 % had anomalies of the sex chromosomes and 15.6 % had autosomal aberrations [32].

Other aberrations

Among other chromosomal defects accompanying sterility is Robertsonian translocations, which include the exchange of whole arms of chromosomes and the reciprocal translocations, which include the exchange of fragments of chromosomes. Both translocations happen 8.5 times more in the population of sterile men in comparison with a randomised group of newborns. Independently on the chromosomes involved, sperm of men with Robertson translocation have pathological characteristics. In the case of reciprocal translocations phenotype varies from normal sperm characteristics to azoospermia [2, 44, 45].

Cystic fibrosis

About 10 % of all congenital azoospermia is caused by the mutations of genes of Cystic Fibrosis (CF) [18, 25, 31]. CF is a recessive autosomal disease, caused by mutation in the gene, which codes the protein responsible for trans-membrane conductance of electrolytes in epithelial cells (cystic fibrosis transmembrane conductance regulator, CFTR).

Gene CFTR was identified in 1989 and today there are about 1000 described mutations, which causes different manifestations of CF. The majority of mutations are very rare. The most common mutation is called ДF508. The frequency of CF varies in different ethnic groups. In a caucasion population one of 25 men carries ДF508 mutation and one of 2000–2500 suffer CF [20].

The classic clinical picture of CF includes progressive obstruction and infection of the respiratory tract, exocrine defects of pancreas, and anomalies of the sugar level in blood. Congenital bilateral absence of vas deference (CBVAD) is an important part of the phenotypic spectrum of disease. 1–2 % of all sterile men have this defect. Among other symptoms of insufficiency of the genitals are hypoplasia, functional insufficiency of the seminal vesicles and ejaculatory ducts, and underdeveloped, firm and swollen epididymis. Some level of spermatogenesis is taking place, albeit abnormally, in patients with CF, and sperm could be obtained by MESA for the IVF-ICSI cycles [39]. It was shown also that gene CFTR is involved in the regulation of spermatogenesis. For example, 16.8% of sterile men, with pathological characteristics of sperm and normal anatomy of reproductive organs (no symptoms of CF) carry CFTR mutation [20].

How CFTR mutation is inherited is not yet clear. Theoretical probability of transmission of this mutation is 50 %. Nevertheless, Josserand et al. [21] describes a group of 50 men with absence of vas deference bilaterally, 41 of which carried the CFTR mutation. Application of MESA with ICSI allowed the birth of 10 children, only 2 of which did not have CFTR mutation, and 8 (80 %) children were born with the same genetic defect as their fathers.

Another concern regarding the mechanism of inheriting CF is that the CFTR mutation is recessive, and 52 % of hetero-zygote carrier manifest symptoms of the disease [21]. It could be explained by the fact that many kinds of this mutation are not yet known, which in the case of manifestion of CF symptoms, homo, but not heterozygote mutations take place.

Micro deletions of Y-chromosome: Azoospermia factor (AZF)

Until recently, the dominant opinion was that the determination of sex, controlled by SRY gene, is the unique function of the Y-chromosome. This theory was radically changed after the discovery of many genes of Y-chromosome, which control spermatogenesis, every mutation of which could lead to sterility [1].

Role of zone of AZF in reproduction

In 1976 Tiepolo and Zuffardi discovered the deletion of one zone of the long arm of Y-chromosome (Yq11) in sterile men, and suggested that this zone could be responsible for the factor of male fertility [46]. This deletion was found in 6 men with azoospermia, which is why it was called Azoospermia Factor Region (AZF). Genes of this zone, code proteins regulating spermatogenesis. Locuses AZFa, AZFb, AZFc, and, some authors believe, AZFd were determined to depend on their location in the long arm of Y-chromosome [17, 46, 49, 50, 51].

It was shown that among all locus of AZF mutations of zone AZFc occurred more frequently. Mutations of AZFa and AZFb are more rare. Nevertheless, deletion of one, two or all three locuses leads to severe disorders of spermatogenesis such as atrophy of testes, hypo-spermatogenesis, and/or blockage of gamete maturation, and others.

Microdeletion of zone AZFc leads to a considerable decrease of spermatogenesis with variable but almost always very low levels of spermatozoa production [34, 37]. Foresta et al [17] review data from different studies of patients with deletion of AZF (1992–2000). 83 % of carriers of deletions of zone AZF suffer azoospermia, 14.1% of them have severe oligozoospermia and only in 1.6% of the cases was sperm concentration higher than 5 x 10^6 /ml. None of the carriers of AZF deletions had normal spermatogenesis.

It was shown that AZFc deletion usually does not lead to the complete arrest of spermatogenesis, and with a testicular biopsy some gametes could be obtained. In combination with ICSI this technique makes it possible for men with AZFc to have children.

Inherit transmission of sterility caused by AZF deletions

The majority of men with deletions of the Yq arm have mutation de novo [34, 35]. Oates et al. [34] demonstrate that none of the fathers of men with AZFc deletions have the same defect. It could be easily explained, because majority of men with deletions of Yq arm are not able to have children without artificial reproductive technologies. In the past this mutation was genetically mortal and the frequency of this defect did not increase in generations to follow. On the contrary, introduction of ICSI into daily practice will lead to increase of sterility in human population [26, 22, 38].

Mutation in the AZF zone happens during meiosis in spermatogony of the father of the sterile male, which leads to the line of spermatozoa, one of which fertilizes the oocyte

and transmits deletion to son. Then, if the man with the AZF deletion becomes a father, all of his children will carry the same deletion. Oates et al. [34] demonstrate that all 18 boys conceived with ICSI from AZFc deleted fathers have the same deletions, which is the reason for the prognosis of sub-fertility or sterility in the future. Cram et al. [9] represents a study with PCR screening of 22 markers of men with AZFc and AZFd deletions and their sons. Results show a stable vertical transmission of AZFd and AZFc deletions in all father-son pairs, which means that all boys conceived with ICSI from men with AZFd and AZFc deletions have the same genetic defect.

Another study [35] represents analysis of three families with AZFc deleted fathers, which generated sons with ICSI. All four boys (one pair of twins) have the same genetic defect as the father.

Thus, not all cases of azoospermia or severe oligozoospermia are caused by AZF deletions, but all carriers of AZF deletions suffer severe spermatogenesis disturbances. Taking into consideration that sons of men with AZF deletion inherit that defect in 100 % of cases, we can conclude that all these population will suffer azoospermia or severe oligozoospermia.

How often does it happen that sterility, caused by AZF deletions, is transmitted to children conceived in centers of artificial reproduction? Foresta et al. [17] summarizes data from research completed at several different centers during 1992–2000. Their results indicate 8.2 % of all patients with severe disturbances of spermatogenesis have deletions in the AZF zone. 14.3 % of patients have idiopathic oligozoospermia (sperm count $< 5 \times 10^6$ /ml), 18 % of patients have idiopathic azoospermia, and 34.5 % of patients with Sertoly Cells Only syndrome have AZF deletion.

Some authors state that 3.8 % of all men who pursue artificial reproduction, carry AZF deletions. It should be noted that men with severe oligozoospermia or azoospermia are the most probable clients of ICSI which is why a portion of carriers of AZF deletion in all population of candidates for ICSI could be much higher.

Male sterility connected with X Chromosome: Repeating CAG and Kennedy disease Among other genes, the X chromosome contains 8 exons, which codes androgen receptors. Exon 1 of androgen receptor contains the zone for repeating triplet CAG, the length of which varies normally from 11 to 31 repeating units. Androgens are the key mediators of normal sexual development in males and play a role in maintaining sexual characteristics and fertility. Androgen receptors are one of the nuclear receptors responsible for the development and homeostasis of embryogenesis. Mutation of the androgen receptor gene could lead to different disorders in the male, among which are syndrome of insensitivity to androgens, cancer of prostate and Kennedy disease [10].

Kennedy disease, also known as spinal and bulbar muscular atrophy, is a fatal X-linked neuro- degenerative disease, that occurs when the androgen receptor gene CAG repeat number is > 40 [30]. Patients with Kennedy disease also manifest the progressive insensitivity to androgens, severe oligozoospermia, and atrophy of testes and gynaecomastia.

The percent of persons with prolonged zone CAG is significantly higher in the population of men with “idiopathic” azoospermia or severe oligozoospermia in comparison to fertile men. Study of Dowsing et al. [14] demonstrate that in a majority of fertile men the number CAG repetition is not > 21, and in men with idiopathic sterility this number is >20 and in some cases may reach 34.

Like all related CAG repeat neurodegenerative disorders, Huntington’s disease, (dentato-rubro-pallidoluysian atrophy, DRPLA) and spino-cerebellar ataxia, the length of the androgen receptor CAG tract shows a tendency to expand over generations leading to an increase in the severity of pathology of Kennedy disease and, a concomitant decrease in the age of disease onset [8]. Moreover, in all related CAG repeat disorders paternal trans-

mission leads to a more intensive increase of CAG tract length over generations, in comparison with maternal transmission.

The length of zone CAG increases during spermatogenesis, which is why the CAG repeat in spermatozoa is considerably bigger in comparison with somatic cells of men-carriers [43]. Application of ICSI for men with increased CAG zone, already long enough to cause sterility, can lead to an increase of CAG repeat leading to an increase in the severity of symptoms of Kennedy disease in the next generation.

CONCLUSION

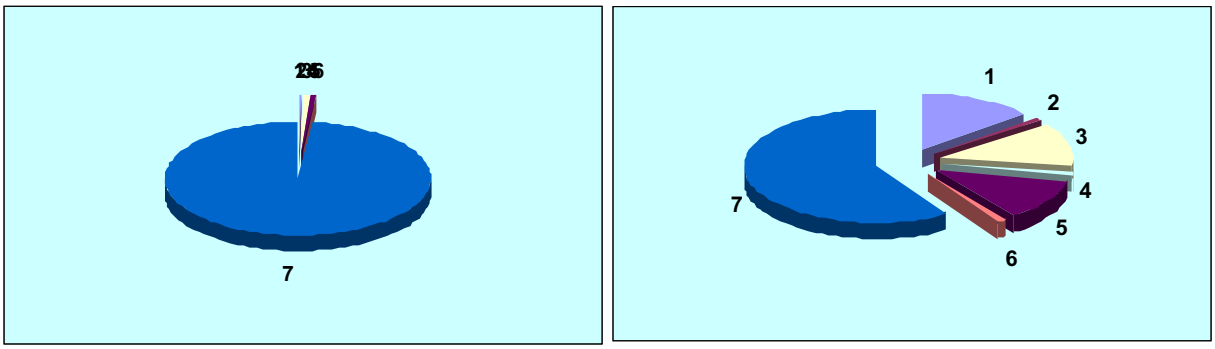
Application of ICSI in cases of genetic male sterility can lead to transmission of sterility and other disorders in children and increasing the percent of sterility in the general human population. About 2 % of all sterile men suffer azoospermia. Faddy et al.[15] show that if half of azoospermic men will refer to ICSI, the percent of severe male sterility will double in the next 7 generations (about 200 years). If 90 % of them refer to ICSI, the frequency of sterility will increase almost two times (1.9 %) during one generation and will increase to 6.7 % over the next ten generations.

Table 1 and figure 1 shows a summary of the percent of genetic and chromosomal anomalies in the population of men with azoospermia and severe oligozoospermia in comparison with the general population. All diseases represented are connected with severe disorders of spermatogenesis, which is why it could almost never be transmitted to children by natural conception. Application of artificial reproductive technologies opens the door for the birth of children with high, sometimes 100 %, chance of receiving inherit genetic defects. Some diseases include only disorders of reproductive function. In these cases the odds of giving birth to a sterile child is increased.

Dangers for children conceived with ICSI from sterile men are not limited to the possibility of inherited genetic or chromosome defects. Disorders of spermatogenesis, independent of causes, leads not only to a decrease in the amount of gametes, but also a decrease in the quality of meioses, when the wrong distinction of chromosomes in spermatocites leads to creation of aneuploid spermatozoa with “de novo” aberrations.

Percent of the most frequent genetic anomalies in the population of men with azoospermia and severe oligozoospermia in comparison with general population

Disease or genetic defect	Disease or defect in general population (%)	Disease or defect in the population of men with severe oligozoospermia (%)	Disease or defect in the population of men with azoospermia (%)	The risk of inherit transmission (%)	References
Klinefelter’s syndrome	0.2	0.7	14	3 – 13.2	7; 40; 42
Klinefelter’s syndrome, mosaic	0.03 (15% of all Kl)		1.4	70	4
CTRF mutation	1	6.6	11.7 –16.8	50 ; >80	11; 16, 19, 20
Dislocation of Robinson		1.6	0.09		3 , 20
AZF mutation	0.4	8	14–35	100	16, 17
Mutation of AR gene	0.05		1.1	100 in holdes 50 in male grandchildren	14;16; 28
All chromosome aberrations	0.3	5.8	13.7	1.3	16; 28; 47



General population Men with azoospermia

Percent of most frequent genetic anomalies among men with azoospermia in comparison with general population. 1) Non mosaic Klinefelter's syndrome; 2) mosaic Klinefelter's syndrome; 3) CFTR mutation; 4)dislocation of Robinson; 5) AZF mutation; 6) AR mutation; 7) normal geno- and karyotype

Klinefelter's syndrome, mutation of gene CFTR (cystic fibrosis) and mutation of AZF zone of Y-chromosome are among the most frequent genetic causes of severe oligozoospermia and azoospermia. The probability of a mutation of the CF gene being transmitted to the next generation is 50 % The probability of inherit transmission of Klinefelter's syndrome of mosaic karyotype could even reach 70 %. The probability of transmission of AZF mutation generated with ICSI, is 100 %. The percent of men AZF mutation among users of ICSI centers, ranges from 3.2 % to 14 %. It means that at least 3.2 % of all boys conceived with ICSI will be sterile because of mutation in AZF zone of Y-chromosome.

In the case of deletion of AZF zone there is certainty that the son will have this defect. In this case the role of counselling is to provide this information to possible parents and discuss with them their feelings and responsibility for the birth of a son who is sterile.

It should be noted that genetic analysis and counselling do not always occur before the beginning of artificial fertilization cycles [24]. Parents-to-be do not always have an opportunity to learn about the genetic risks of their unborn child and make any subsequent and responsible decisions, among which is that of not to resort to ART, but to live own fertility in the other way. Responsibility for high risk of conceiving a child with genetic anomalies rests not only with the parents, but with all of society to include those responsible for research, technology, and legislation, in the health care profession.

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TRANSMISSION OF MALE INFERTILITY AND INTRACYTOPLASMIC SPERM INJECTION (MINI-REVIEW)

The fact that genetic aetiology is responsible for approximately one third of all kinds of male sterility has led to concerns regarding the application of artificial reproductive technologies in the cases of azoospermia and severe oligozoospermia. Congenital sterility could be caused by gene mutation, quantitative or structural abnormality of sexual chromosomes or autosomes. The possibility of inherited transmission of male sterility within the context of applying artificial reproductive technologies is analysed in the article. Klinefelter's syndrome, mutation of cystic fibrosis transmembrane conductance regulator (CFTR) gene, which causes cystic fibrosis, and mutation of azoospermia factor zone (AZF) of Y-chromosome are among the most frequent genetic causes of severe oligozoospermia and azoospermia. The probability of a mutation of the CFTR gene being transmitted to the next generation is 50%. The probability of inherit transmission of Klinefelter's syndrome of mosaic karyotype could reach 70%. The probability of transmission of AZF mutation to children of male sex, generated with ICSI, is 100%. The percent of men with AZF mutation among users of ICSI centers, ranges from 3.2% to 14%. It means that at least 3.2 % of all boys conceived with ICSI will be sterile because of mutation in the AZF zone of Y-chromosome. It should be noted that genetic analysis and counselling do not always occur before the beginning of artificial fertilization cycles. Parents-to-be do not always have an opportunity to learn about the genetic risks of their unborn child and make any subsequent and responsible decisions. Among

the decisions is a choice of not resorting to ART, but to live with the infertility and explore other opportunities for parenthood. Responsibility for high risk of conceiving a child with genetic anomalies rests not only with the parents, but also with all of society, including those responsible for research, technology, and legislation, in the health care profession.

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