Genetic aspects of infertility and miscarriage – a review of literature

A. M. FESKOV, S. B. ARBUZOV, V. V. GRABAR

Abstract
We present current data on the disturbance of reproductive function in men and women according to genetic causes. The purpose of this paper was to discuss the influence of heredity on sexual differentiation, gametogenesis, early stages of embryogenesis and implantation.

Key words: infertility, heredity, pregnancy, miscarriage

Introduction
Despite the achievements of clinical medicine up to half or more cases of infertility, habitual miscarriage, causes of violations of reproductive function remain unclear [3, 35].

Modern reproductive technologies solve the problems of infertility in many ways. However, treatment of infertility can be considered effective and justified not only upon the occurrence of pregnancy, but also in problem-free course and getting a healthy, full-fledged offspring [25, 30]. Therefore, the assessment of genetic risk factors and the search for methods of diagnosis of genetic diseases are of paramount importance [10, 14].

Genetic factors influence all stages of development and functioning of the reproductive system. Genetic factors that may disrupt reproductive function include chromosome abnormalities (numerical and/or structural), mutations of genes controlling reproductive function (Y-linked, X-linked, autosomal, mitochondrial), mosaicism, different DNAs and chromosome polymorphisms as well as epigenetic factors [4]. Therefore, infertility may be associated with various disorders of sexual differentiation, gametogenesis (spermatogenesis and oogenesis), defects of intergametic interaction and fertilization, violations of the early stages of embryogenesis, and implantation [1, 11, 33]. It should be also considered that a large part of the endocrine dysfunction (defects in the synthesis of hormones, realization of their function) and immunological disorders (inadequate immune response) can be attributed to the influence of genetic factors that may be their primary cause or a favorable background for their development [7, 13].

Chromosomal abnormalities
Sexual differentiation is a sequential and orderly process. Chromosomal sex, emerging at the time of fertilization determines gonadal sex and that in turn determines the development of phenotypic sex, involving the formation of male and female sexual apparatus. Changes at any stage of this process during embryogenesis lead to damage of sexual differentiation [11, 17, 28].

The first stage of sex differentiation establishes chromosomal sex: male (XY heterogamete) and female (XX homogamete) [1]. Up to 40 days of gestation embryos of both sexes develop identically. The second stage of sex differentiation is the transformation of undifferentiated gonads into testes or ovaries.

The differentiation of gonads in the testes is mediated by genes of the Y chromosome. The final process - broadcast of gonadal sex into phenotypic sex - depends on the type of fetal gonads and their endocrine secretion. The development of phenotypic sex leads to the formation of male and female urinary system [34].

Klinefelter’s syndrome
The abnormalities of chromosomal sex are Klinefelter’s syndrome, males with XX karyotype, gonadal dysgenesis (Turner’s syndrome) and mixed gonadal dysgenesis [26, 28]. Klinefelter’s syndrome is the most common damage of sexual differentiation (1:500 males) with 47,XXY karyotype in the classic form or 46,XY/47,XXY in mosaicism. The classic form is caused by nondisjunction of chromosomes in the process of gametogenesis. About 40% cases of nondisjunction in meiosis occurs in spermatogenesis, 60% – in oogenesis [11, 33]. With increasing age of the mother, the probability of nondisjunction increases. The mosaic form is due to nondisjunction of chromosomes in mitosis after fertilization [24]. Klinefelter’s syndrome is characterized by primary hypogonadism, azoospermia due to hyalinization of seminiferous tubules, gynecomastia, and elevated gonadotropins in the human plasma [15].
The study of chromosomal karyotype in peripheral blood leukocytes revealed that a mosaic variant affects about 10% of patients. The frequency of this variant is likely underestimated, as chromosomal mosaicism can occur only in the testes, while the karyotype of peripheral leukocytes remains normal. The mosaic form is generally not shown as hard as variant 47,XXY and the testes may remain normal in size [20]. Endocrine disorders are also less pronounced, and gynecomastia and azoospermia are less common. Very rarely, patients with mosaicism preserve fertility. Restoration of fertility in Klinefelter’s syndrome is impossible [31].

**De la Chapelle syndrome**

De la Chapelle syndrome (XX male) in the phenotypic males occurs in 1:20000-1:24000. Yet female genitalia are absent, in psychosocial terms, these individuals feel that they are men. Phenotypic characteristics of the syndrome are similar to those with Klinefelter’s syndrome: hypogonadism, azoospermia due to hyalinization of seminiferous tubules, low plasma testosterone level, increased concentration of estradiol and increased content of gonadotropins. The height of these patients is on average lower than in Klinefelter’s syndrome [16, 28]. The pathogenesis of the disorder is currently due to the following mechanisms: translocation of a part of Y chromosome on X chromosome, mosaicism on Y chromosome in some cell lines or an early loss of Y chromosome, autosomal gene mutation, a deletion of X-chromosome genetic material, normally providing a negative regulatory effect on the development of the testes [5].

**Gonadal dysgenesis**

Gonadal dysgenesis (Turner’s syndrome) is characterized by primary amenorrhea, sexual infantilism, dwarfism, the presence of gonadal strands in phenotypic women with a defect in X chromosome. Approximately 50% of patients show 45,X karyotype, 25% – mosaicism without structural defects (46,XX/45,X), and the rest – structural damages of X chromosome with or without mosaicism. Option 45,X is due to a chromosome loss during gametogenesis in either parent or a violation of mitosis in one of the early divisions of the fertilized zygote [17]. Dwarfism and other physical changes are due to the eventual loss of genetic material from the short arm of the X chromosome; gonadal strands are formed in the result of the loss of genetic material, either from a long or a short arm of the X chromosome.

In patients with mosaicism or structural disorders of the X chromosome, changes in the severity of the phenotypic type occupy an intermediate position between those observed in the form of 45,X and the norm.

Some patients with macroclitoris, in addition to the X chromosome, possess a fragment of a chromosome, presumably – abnormal Y chromosome. In rare cases, a balanced X-autosomal translocation may cause the family transfer gonadal dysgenesis [8]. The frequency of gonadal dysgenesis is 1 : 2500 of newborn girls. Gonadal dysgenesis is the most common cause of primary amenorrhea, patients do not reach sexual maturity and feminizing type external genitalia are undeveloped.

In the process of embryogenesis, transiently appear primordial germ cells, but they disappear as a result of accelerated atresia. By the age of expected puberty, these hands do not contain distinct follicles and oocytes, but they possess fibrous tissue, indistinguishable from normal ovarian stroma.

In rare cases (2% compared with the classic version 45,X and 12% with mosaicism), in the ovaries, the number of follicles remains sufficient to arise rare menses, but pregnancy is rarely possible. However, the duration of fertile period in these women is not long. A link between fertility in women with Turner syndrome and the structure of the X chromosome is probable.

Perhaps the genes needful to maintain the functional capacity of oocytes, are localized between Cr21 and the centromere [32]. Mixed gonadal dysgenesis – a condition in which a phenotypic men and women on one part have testicle, and on the other – gonadal strand.

The majority of patients presents with mosaicism 45,X/46,XY. The cause of mosaicism best accounts for the loss of Y chromosome in the early stages of XY-zygote mitotic division. According to most authors, this is the second most common cause of genital ambisexuality to congenital adrenal hyperplasia [19, 29].

**Hermaphroditism**

True hermaphroditism is a condition in which the patient has both the ovaries and testes or gonads with histological features of either sex (ovotestis). The following 3 options can occur: 20% possess ovotestis on both sides, 40% possess ovotestis on one side and either the ovary or the testis on the other side, and 40% possess testis on one side, and ovary on the other side [16, 34].

External genitalia of these patients are at different stages of transition from male to female. Approximately 60% patients have 46,XX karyotype, 10% – 46,XY, and the rest chromosomal mosaicism, in which there is a cell line with a Y-chromosome.
It is believed that in this case there is sufficient genetic material of Y-chromosome to induce the development of testicular tissue [28]. There is evidence of familial forms of true hermaphroditism with 46,XX karyotype, due, probably, to the presence of an autosomal recessive gene or a common translocation [10]. Since the ovaries of more than 25% of patients contain yellow bodies, we can conclude that such individuals possess normally functioning female neuroendocrine system [24]. Feminization (gynecomastia and menstrual period) is due to ovarian secretion of estradiol.

In masculinized individuals, secretion of androgen prevails over secretion of estrogen, and some of them produce sperm [16]. The damages to gonadal sex include pure gonadal dysgenesis and the syndrome of absence of the testicles.

**Pure gonadal dysgenesis**

Pure gonadal dysgenesis (Swyer’s syndrome) – is a disorder in which individuals with female phenotype are of normal height, normal 46,XX karyotype or 46,XY, but the state of their genital organs corresponds to that in gonadal dysgenesis (bilateral gonadal strands, infantile uterus and fallopian tubes, sexual infantilism).

It is impossible to clinically differentiate pure gonadal dysgenesis and gonadal dysgenesis (Turner’s syndrome) with minimal somatic abnormalities. Estrogen deficiency varies from sharply pronounced characteristic of the typical 45,X-gonadal dysgenesis, to a minor.

Patients with varying degrees of development of mammary glands, have menses, though menopause comes early. Both XX and XY-options may be due to mutation of a single gene. The mutation is assumed to be transmitted as coupled with the chromosome recessive trait [17, 34].

**Absence of testicles**

The syndrome of absence of the testicles is found in individuals with a 46,XY karyotype who are missing or possess rudimentary testes, but at some stage of intrauterine life, unmistakable signs of endocrine function of these glands appear (occurs regression of Mullerian ducts and secretion of testosterone). Clinically, the syndrome manifests in varying degrees – from a complete lack of virilization, incomplete virilization of the external genitalia to a normal male phenotype, except for bilateral anoschism syndrome in patients with male phenotype are also marked out. The pathogenesis of the disease is not fully understood, multiple family cases suggest that the cause is associated with the presence of the mutant gene [18].

The damages to phenotypic sex, which lead to female pseudohermaphroditism include congenital adrenal hyperplasia and congenital defects of Mullerian ducts (Mayer-Rokitansky-Küster-Hauser Syndrome).

**Mutation of genes**

Congenital adrenal hyperplasia (adrenogenital syndrome) is a group of autosomal recessively inherited disorders of synthesis of corticosteroids. More than 90% of all cases of adrenogenital syndrome are due to the lack of CYP21A2, a gene which is located on the short arm of chromosome 6 near the locus HLA-B. Frequency of CYP21A2 deficiency is 1:50. Pathogenetic entity of adrenogenital syndrome is a damage to production of hydrocortisone; compensatory increasing secretion of adrenocorticotropic hormone causes hyperplasia of the adrenal cortex and the secondary increasing of androgen secretion, causing virilization in women and premature masculinization in males [17, 34]. Carriers of the disease can be identified by the haplotype HLA.

**Female pseudohermaphroditism**

Female pseudohermaphroditism may be caused by lack of CYP11B1. Clinical manifestations due to glucocorticoid deficiency and excess of androgens are similar to those in CYP21A2 deficiency; this form is characterized by hypertension [8].

**Mayer-Rokitansky-Küster-Hauser syndrome**

Mayer-Rokitansky-Küster-Hauser syndrome as a cause of primary amenorrhea is second only to gonadal dysgenesis. In the majority of patients, the syndrome is diagnosed in the expected age of puberty as a result of absence of menstruation [17, 34]. These women have a normal female phenotype, standard-size uterus, devoid of external channel, but more often represented by two-horned strands. Ovulatory peak level of luteinizing hormone indicates safety of ovarian function and high density of estradiol in plasma.

30% present with kidney abnormalities, frequently agenesis or ectopia, 10% of patients present with damages of the skeleton, most often affected spine, rarely – extremities and the ribs. All patients possess 46,XX karyotype, most cases are sporadic, although cases of autosomal dominant sex-limited mutations family history are also known [4].
**Male pseudohermaphroditism**

Damages to the phenotypic sex, showing male pseudohermaphroditism may be due to a damage to androgen synthesis or androgen action. In 80% of cases of male hermaphroditism, synthesis of androgens in patients remains normal [8].

Five enzyme defects are currently in use, leading to a damage to the synthesis of testosterone and causing incomplete virilization of male fetus during embryogenesis. All these enzymes catalyze the conversion of cholesterol to testosterone at certain stages. Lack of these hormones leads not only to male hermaphroditism, but also to congenital adrenal hyperplasia. Clinical manifestations in these patients may vary from phenotypic males with mild hypospadias to phenotypic women. Most of these conditions are an autosomal recessive trait [16].

**Testicular feminization**

Testicular feminization syndrome (TFS, androgen insensitivity syndrome), also referred to as androgen resistance syndrome, is a set of disorders of sexual differentiation caused by mutations of the gene encoding the androgen receptor. The androgen insensitivity syndrome has been linked to mutations in “AR”, the gene for the human Androgen Receptor, located at Xq11-13 (i.e. on the X chromosome) [29]. Thus, it is an X-linked recessive trait, causing minimal or no effects in 46,XX people.

TFS is characterized by 46,XY karyotype, the gonads and internal genitalia of male-type, wide variability of the structure of the external genitalia from the female regular structure in full form of TFS to intersex or correct male structure in incomplete form of TFS [28]. In embryogenesis, AR regulates the proper development of Wolffian ducts and the male pattern formation of the external genitalia; in puberty – the development of secondary sexual characteristics and spermatogenous epithelium maturation [16]. Thus, the AR protein mediates the effect of androgens on intracellular mechanisms responsible for the development of male sexual characteristics and fertility [35].

**Defects of gametogenesis**

Damages to gametogenesis may be a consequence of altered gene carriers, chromosomal disorders in the spouses, which results in gametes with unbalanced chromosome sets [3, 14].

**Oogenesis**

Oogenesis can be damaged at deletion in the localization of aromatase CYP19A1 gene. In this case, the lin-ks of ovarian estrogen biosynthesis undergo to damage, there is a reduced sensitivity to the influence of ovarian follicle-stimulating hormone, decreased anlage of antral follicles [10].

**Spermatogenesis**

Spermatogenesis is a complex multistep process culminating in the formation of mature sperm. This process is controlled by a large number of genes located both on autosomes and on gonosomes (sex chromosomes), especially on Y chromosome [15, 30]. Gene mutations that control the stages of spermatogenesis may lead to both mobility and morphological properties of fertile sperm damage, blocking of spermatogenesis, manifesting in a range from mild reduction of spermatopoietic activity to the total absence of germ cells in the seminal tubules [5].

One of the most frequent genetic causes of male infertility is a mutation in one of the loci of Y chromosome (AZF-locus, Azoospermia Factor).

AZF microdeletions of the chromosome Y locus are found on the average of 10-15% of cases of azoospermia and 5-10% of severe oligozoospermia and result in a disturbed spermatogenesis and infertility in men [15].

AZF-locus contains three non-overlapping sub-regions: AZFa, AZFb and AZFc [25]. Each of them contains a number of candidate genes, mutations of which lead to azoospermia or severe oligozoospermia. However, the gene (or genes) responsible for the emergence of this pathology of spermatogenesis is not defined [18].

Genetic changes may be also responsible for sperm motility. When accompanied by the combination of situs inversus (reversal of the internal organs), chronic sinusitis, and bronchiectasis, it is known as Kartagener syndrome, if not – the last two symptoms of the triad are meant. Most patients with the Kartagener syndrome demonstrate severe asthenozoospermia. So far, attempts to map the single gene for the Kartagener syndrome failed, most likely there is polygenic trait determination of ciliary motility. One reason for the blocking of spermatogenesis can be balanced autosomal anomalies (translocations, inversions) [11].

**Defects of gametes transport**

In some hereditary syndromes, transport of gametes can be disrupted [16]. One of the commonest causes of excretory infertility is a congenital obstruction of vas deferens due to cystic fibrosis, which occurs in 1-2% of infertile men [19]. The main reason for cystic fibrosis is gene mutations of CFTR protein (cystic fibrosis transmembrane regulator). More than 95% of men with cystic
fibrosis present with obstructive azoospermia. Most have bilateral congenital obstruction of proximal deferent duct or the epididymis.

Azoospermia

Testicular histology usually shows preserved spermatogenesis. Minor mutations of the gene CFTR protein do not show classical picture of cystic fibrosis, only obstructive azoospermia occurs [6]. Obstructive azoospermia is a condition of Young’s syndrome symptom complex. Azoospermia in Young’s syndrome can occur in previously fertile patients [5]. This suggests that the occlusion develops gradually in the initially passable epididymis, probably due to the development of condensed secretion. Young’s syndrome is characterized by the absence of structural anomalies of the vas deferens, epididymis and seminal vesicles. The volume of ejaculate and the content of fructose in it remain normal, which is another difference from cystic fibrosis [11]. Testicular biopsy reveals a picture of stored sperm, but usually there is also impaired motor function of sperm and the level of sex and gonadotropic hormones is not changed [18].

Defects of embryogenesis

Damage to the early stages of embryogenesis occurs in chromosomal abnormalities in the embryo, a prerequisite of which may be qualitative and quantitative chromosomal abnormalities, mosaicism in parents, or de novo mutation in their gametes [14].

It is assumed that many developmental disorders with chromosomal aberrations in the embryo are connected with a reduced ability of cells to divide. Thus there is a sudden desynchronization of embryonic development, the development of the chorion and induction of differentiation and migration of cells [27]. The death of the embryo is not due to a damage to embryonic development (the detectable defects themselves can not be the cause of death of the embryo), but a damage to the formation and functioning of the chorion [7].

Investigation of cell lines in newborns with trisomy 13, 18, 21 showed that the cells divide more slowly than in the normal karyotype, which is manifested in the reduction of the density of cells in most organs [1].

Significant role in the damages to the pre- and post-implantation stages of ontogeny belongs to chromosome mosaicism [21]. The presence of a high frequency of mosaic karyotypes indicates that, along with errors of mitotic segregation of chromosomes, damage to the distribution of mitotic chromosomes in somatic cells of the embryo is of the essence in the occurrence of fatal genomic mutations.

However, cytogenetic and molecular mechanisms of induction of chromosome nondisjunction and, as a consequence of mosaicism in the embryonic period of ontogeny remain unclear. The maximum accumulation of damages to mosaic karyotype falls on the stage of crushing of blastomeres.

The average frequency of mosaicism in embryos is about 26%, while in embryos with developmental arrest frequency of mosaicism is 37%, in normally developing embryos – 25%. In the presence of fragmentation or multicore blastomeres frequency of mosaicism makes up 44% [14].

Damages to the early stages of embryogenesis may occur as a result of epigenetic processes [27]. The epigenetic processes refer to heritable, stable, but potentially reversible changes in gene expression not related to damages to their nucleotide sequence. Molecular basis of epigenetic phenomena are modifications of chromatin structure. Epigenetic phenomena are responsible for the implementation of some genetic processes such as differential gene expression, genomic imprinting, X-chromosome inactivation and repression of mobile genetic elements [23].

Until now, remains unknown contribution of abnormal epigenetic modifications of the genome in damage to the embryonic period of ontogeny, which in humans is characterized by a high frequency of reproductive loss – about 60% of zygotes is eliminated at the pre- and early postimplantation stages of development; 15-20% of clinically recognized pregnancies are spontaneously interrupted pending the first trimester [11]. Genomic mutations are dominant factor causing early inhibition of embryonal development in humans, represented mainly by numerical chromosome disorders [7]. With regard to in utero dead fetuses with normal karyotype, the genetic causes of the termination of their development are usually unknown.

Epigenetic factors of sperm are functionally consistent centrosome, proper packaging of chromatin with protamines, histone modifications and imprinting of genes [23].

In the process of maturation of sperm, a significant reorganization and condensation of the genome occurs. During this phase, most histones acetylize and then replaced with protamines, which shrink a gene of the sperm in the nucleus in a special structure that can serve as an important epigenetic factor for the embryo [25].

It has been found that the anomalies in the organization of chromatin of sperm nuclei (abnormal chromatin compaction) affect the early development of embryos in
IVF programs, and frequency of pregnancy. It is known that the expression of the male genome occurs in human embryos at stage 4.8 blastomeres [1]. It is possible that abnormalities in the mechanisms controlling the compaction of chromatin, affect the early stages of embryo development, which displays in time dilation of fragmentation in the culture [27].

Centrosome of the sperm penetrates the oocyte at fertilization and forms a star, consisting of radially oriented microtubules that contribute to the pronuclei connection and the formation of the spindle in the first mitotic division of zygote. Defects in the centrosome may lead to damaged fertilization and embryonic developmental arrest due to the formation of abnormal spindle and the accumulation of abnormal cells [11, 21]. In addition, the early stages of embryogenesis can be influenced by characteristics of oocytes.

It has been found that the efficiency of mitochondrial respiration in oocytes and embryos is closely correlated with the programmed rate of embryo development, and in addition to that, a woman’s age significantly influences the activity of mitochondria [33]. Reduced activity of mitochondria in the oocytes of older women may explain the lower incidence of embryos and pregnancy in IVF cycles [7].

**Defects of implantation**

According to current data, damage of implantation and miscarriage is regarded as multifactorial pathology, which develops as a result of combined effects of functionally attenuated variants (alleles) of sets of genes and adverse external and internal factors [3, 9, 12].

The relative function of each genetic and environmental factors varies in each case. Thus, a link between damages of implantation and miscarriage with a functional weakening of the exchange of folate genes (MTHFR, MTRR), system detoxification genes (GSTM1, GSTT1, GSTP1), the genes of HLA (DQA1, DQB1, DRB1, HLA-G), the genes of growth factors (VEGF), endothelial dysfunction genes, blood-coagulation factors genes (FI, FII, FV) [11, 18, 34] is established.

Defects of implanted fetal eggs, which take place in conditions such as antiphospholipid syndrome, genetically mediated hypofibrinolysis (polymorphisms in the genes of plasminogen activator inhibitor, tissue plasminogen activator) and hyperhomocysteinemia (mutation in the MTHFR, MTHFD, MTS, MTRR genes) are the cause of early preembryonic loss [12]. In a genetically caused hypofibrinolysis and activation of intravascular coagulation, occurs desynchronization of fibrinolysis and fibrinogenesis during implantation [2]. Under these conditions, the activity of proteases synthesized by blastocyst, becomes relatively insufficient to destroy the extracellular matrix in the endometrium and to penetrate to a sufficient depth.

If followed by the circulation of antiphospholipid antibodies (according to various sources 40-60%) [9], it exacerbates the situation since antiphospholipid antibodies are not only increasing prothrombotic mechanisms, and therefore the processes of fibrinolysis and fibrinogenesis, but also are able to alter the surface characteristics of the pre-implantation fetal eggs, both the charge, and configuration [2].

Genetic disorders affect not only the gametogenesis, embryogenesis, implantation, but also the later stages of the gestational process.

It is known that mucosal immunity in cervical canal is also partially innate. Normal gene expression of TLR2 and HBD1 provides sufficient protective properties of cervical mucus, which prevents amniotic infection, and, therefore, early termination of pregnancy [22].

**Conclusions**

To conclude, genetic factors underly the large number of reproductive failures, and should be considered when choosing the optimal way of ART (assisted reproductive technologies) in reproductive health clinics. In addition, the possibility of pregnancy in genetically compromised couples raises the problem of pre-implantation and prenatal diagnosis in pregnancies obtained with the help of ART.

**References**


