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Review Article

Genetics of fertility: Time to Explore

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Abstract

Disorders in human Genetics impact their reproductive potential in several ways such as fertility, retaining fertility and fertility treatment outcomes. However, alterations in genes might be harmless or can bring about detrimental outcomes. Understanding the impact of genetics on reproduction is essential to have better fertility outcomes. An individualized assessment of a couple to identify the underlying aetiology related to genetic problems along with genetic testing is considered the first step of the solution. Moreover, assisted reproductive technologies (ART) are a possible fertility treatment option to address feasible genetic problems even before the establishment of pregnancy. A pre-ART assessment of a couple with potential genetic problems can be carried out with expanded carrier screening and genetic analysis of the gametes and embryo during the ART procedure to detect abnormalities and select a healthy embryo are play an indispensable role to overcome this puzzle.

Keywords

Human genetics, subfertility, Assisted Reproductive Technology (ART)

Introduction

Genetics is the study of genes and the pattern of inheritance of characteristics and diseases. The human genome has 23 pairs of chromosomes that carry millions of genes responsible for various functions in the body. Alterations in genes are sometimes harmless if it occurs without a change in their vital function. On the other hand, alteration in functionally essential genes might cause detrimental outcomes. Chromosomal abnormalities can be numerical or structural. Numerical abnormalities are due to alterations in the number of chromosomes such as missing from a pair is called monosomy (eg: Turner's syndrome) and having an additional chromosome is called trisomy (eg: trisomy 21 is down syndrome). The structure of a chromosome can be altered in several ways such as deletions of a certain portion of chromosomes, duplication and translocations where a portion of the chromosome is transferred to another or inversion of a broken segment of the chromosome is reattached upside down(1). Mutations are alterations in the nucleic acid sequence of the organisms which occur during mitosis or meiosis. Genetic impact on reproduction is essential to understand for better fertility outcomes of couples who suffer from fertility problems such as inability to conceive, recurrent pregnancy loss and recurrent fertility treatment failure. One in seven couples is facing problems with subfertility(2). Subfertility could be due to female factors in 30%, male factors in 30%, both factors in 15 % and the rest 25% is unexplained. Among these, genetic causes are included in all 3 forms of sub-fertility (3). Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies and is recommended to diagnose the possible cause for RPL after the loss of two or more pregnancies. Genetic abnormalities of the conceptus, especially aneuploidy, are a recognised cause of sporadic and recurrent pregnancy loss (RPL). Abnormal parental karyotypes were found in around 1.9% of couples with recurrent pregnancy loss (4).

Genetics and subfertility

Even though there are many non-genetic causes of subfertility, genetic causes have to be considered as one of the key elements to decide the optimal fertility outcome in sub-fertile couples. The genetic disorders resulting in male and female subfertility are chromosomal aberrations, chromosomal microdeletions, single gene disorders and the risk of conveying genetic disease to a child (5).

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1. Male subfertility

1.1 Numerical chromosomal abnormalities

46, XX male is another disorder affecting fertility in males. It is due to the translocation of Y chromosome materials, including genes of the sex-determining region, to the X chromosome, which leads to a male chromosomal complement(6). These men have normal sexual development and the presence of testis, but they do not have spermatogenesis.

Kleinfelder's syndrome is another numerical chromosomal abnormality that causes subfertility and which has many genotypes. The most common karyotype is 47, XYY with a prevalence of 1/1000 (7). They may also present as mosaics where there are normal 47, XY cell lines in addition to pathological 47, XXY and they may be oligospermia.

1.2. Structural chromosomal abnormalities

1.2.1. Y chromosomal deletions

Among sub-fertile couples, the male partner is responsible for 30% of the time. Among them, about 40% of Men have no identifiable cause of subfertility(3). This is said to be "Idiopathic subfertility ", in which most are due to genetic causes (21). 10 – 15% of men with non-obstructive azoospermia are due to Y chromosomal microdeletions. Between 1 and 8% of these microdeletions occur in an area called azoospermia factor (AZF) (8). AZF is further divided into AZF a, b and c. Micro deletions can occur in any of these areas. AZF c is responsible for 60% of all AZF deletions (9). gr/gr deletion is a form of AZF deletion where men tend to have a varying degree of spermatogenesis. Some deletions of the AZF region cause Sertoli cell-only syndrome due to the failure of the development of Sertoli cells that nurture the developing sperms.

1.2.2. Translocations

1.2.2.1. Robertsonian translocations

The other common recurrent chromosomal translocation causing subfertility is Robertsonian translocation. It is due to the fusion of long arms of 2 acrocentric chromosomes. Translocation between chromosomes 13 and 14 is frequently associated with infertility. However, the inherent cause of subfertility in carrier men is not established in the literature(10). These translocations can also occur between group D chromosomes, such as chromosomes 13, 14, and 15 and group G chromosomes, such as 21, 22 and Y) (6).

1.2.2.2. Reciprocal translocation

These are due to breakage and exchange of distal segments between non-homologous chromosomes. The incidence is one in 712 births. It accounts for 1.17% of sub-fertile men. The effects of translocation differ according to the breakpoints and the chromosome involved, and the majority might result in fetal demise.

1.3. Germline mosaics

Germline mosaics are men with normal karyotypes but have abnormal cell lines in their testicular cells which results in subfertility, recurrent pregnancy loss and causing abnormalities in offspring(11).

1.4. Other syndromes associated with male sub-fertility

Mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which presents with the endocrine and respiratory system, causes Cystic fibrosis. It is an autosomal recessive disease. Men with cystic fibrosis have failed to develop normal vas deferens and, in extreme cases, can result in congenital bilateral aplasia of vas deferens which eventually causes subfertility (12).

Kallmann syndrome is a congenital cause of hypogonadotropic hypogonadism. Some cases of Kallmann syndrome are caused by deletions or point mutations in the KISS1R gene, and mutation in KAL genes causes x-linked Kallmann syndrome (11,13). Primary ciliary dyskinesia is an autosomal recessive disorder affecting the motility of cilia of spermatozoa resulting in subfertility(14).

Other rare genetic conditions also cause male subfertility, including Young syndrome, characterised by sinopulmonary infections, persistent azoospermia but normal spermatogenesis, Androgen Receptors mutations resulting in androgen insensitivity syndrome, Kartagener syndrome, metabolic syndrome, polyglandular syndrome, persistent Mullerian duct syndrome, Noonan syndrome and Prader-Willi syndrome(6,15).

2. Female subfertility

2.1. X chromosome and infertility

In human monosomy X (45, XO- Turner syndrome), due to the complete loss of X chromosomes and causing rapid loss of primordial cells during the development and streaky ovaries at birth has resulted in primary ovarian insufficiency (POI)(16). Partial deletions in the chromosome in the forms of Duplications, inversions, complex rearrangements, X-autosome translocations, and single-gene sequence variants result in recurrent pregnancy losses (17,18).

A few sub-fertile women have both the cells carrying the karyotype of 46, XX and 45, XO in their body. They might have a menstrual cycle at a young age followed by early menopause, or they will also present with recurrent pregnancy losses. (16)

2.2. Problems of sexual differentiation

The problems in sexual differentiation results in gonadal dysgenesis. 46XY gonadal dysgenesis is due to the mutation in the SRY gene on the Y chromosome, which is also responsible for the production of anti-mullerian hormone; as a result, Mullerian structures persist. Therefore, a genotypical male will have a female appearance. They have nonfunctioning gonads and delay in puberty (19).

Genetics and miscarriages

Miscarriage occurs at the frequency of 10-20 % of all pregnancies (20). While most of the losses are due to sporadic genetic abnormalities(21). It may also be recurrent which is determined by the nature of the genetic change. Chromosomal abnormalities are responsible for miscarriages in genetically abnormal fetuses, and early pregnancy losses are mostly due to genetic abnormalities (20). Miscarriages could be due to parental or fetal causes.

1. Parental causes of miscarriages

2 to 4% of recurrent miscarriages are due to parental balanced chromosomal translocations. The most common translocation is balanced reciprocal translocation followed by Robertsonian translocations and parental mosaicism, and interstitial microdeletions are rare parental causes of miscarriage (22,23).

Epigenetic changes are alterations in a gene affecting gene activity and expression. In normal females, 50% of paternal X chromosomes and 50% of maternal chromosomes are inactivated. 75-80% of X chromosomes are asymmetrically inactivated and known as X skewed. It is one of the common epigenetic changes resulting in miscarriage (24). Methylation is one of the epigenetic changes essential for the inactivation of a specific gene sequence which helps cell homeostasis, and any alteration here would result in recurrent pregnancy loss (25).

2. Fetal causes of miscarriage

Aneuploidy of the fetus is responsible for a proportion of recurrent pregnancy losses(26). The most common type of aneuploidy causing a miscarriage is trisomy, among which trisomy 16 is the most common. The other types, like trisomy 13, 11 and 28, are common in those having late pregnancy losses and stillbirths (20).

Apart from aneuploidies, a small proportion of deletions in genetic materials is known as Copy Number Changes (CNC). Even though CNC is a less significant cause of miscarriage, changes that occur in physiologically important genetic materials may lead to miscarriages (27).

Single gene disorders seldom cause recurrent pregnancy loss; on the other hand, it causes inborn errors of metabolism and hemoglobinopathies such as thalassemia, sickle cell disease and X-linked diseases, which may also result in recurrent pregnancy losses (20). Prolonged QT syndrome due to channelopathies in the fetus is another genetic disorder that causes sudden cardiac death in fetuses with an anatomically normal heart(28).

Genetics and recurrent implantation failure

Recurrent Implantation failure is the inability of the blastocyst to incorporate into the uterine wall after fertilisation naturally or by artificial reproductive techniques. The genetic causes of recurrent implantation failure are almost the same as recurrent miscarriages. Invasion of blastocyst followed by angiogenesis are essential steps in implantation. Any abnormalities in genes responsible for cytokines and growth factors would result in recurrent implantation failure (29).

Assessment of a couple

The complete assessment of a subfertile couple should include a proper history, examination and investigations.

History

History has an important role to play in sub-fertile couples to exclude the possibility of genetic causes. History should include growth and development, sexual development and exposure to the chemicals and toxins such as alcohol, lead, mercury, and x-rays. Since the genetic causes of subfertility are asymptomatic and running in the families in the majority of couples, the pedigree chart plays an indispensable role in assessing the genetic issues related to fertility problems. This could assist to precede targeting the investigations and plan further management of the couple (30).

Examination

• General examination:

The whole body should be examined for the general features of dysmorphism of syndromes such as Turners, Kleinfelder's and Down's syndromes and relevant general examination features should look related to specific syndrome (Table 1)

Table 1 Clinical features of major syndromes

Down syndrome	Klinefelter's syndrome	Turners syndrome
Learning difficulties	Small testes	Short stature
Hypotonia	Gynecomastia	Neck webbing
Up slanted palpebral fissures, flat nasal bridge, small mouth and ears, protruding tongue, short neck	Tall stature	Widely spaced nipples
Simian crease	Emotional problems	Delayed puberty

• System examinations

All system examinations must be performed, giving special attention to breasts and genitals. A breast examination has to be performed to assess the degree of breast development related to Tanner staging. The genital examination has to be performed in both couples to exclude the syndromes and development assessment, which is relevant to basic subfertility workup.

Investigations

Investigations are the cornerstones for the diagnosis of genetic causes of sub-fertility. Genetic testing can be performed in couples, fetuses and in products of conception following the miscarriage to identify the genetic causes. Those couples with previous exposure to gonadotoxic such as radiation or a history of recurrent previous miscarriage or signs and symptoms of syndrome may need a genetic workup including karyotyping.

Parental genetic assessment mainly includes karyotyping, Y chromosomal micro-deletion testing and CFTR gene mutation identification (31). Karyotyping is the visualisation of prepared chromosomes under a light microscope to detect the numerical and structural alterations. There are sophisticated modern-day tests like genome sequencing to specifically identify defective genes suspected as the cause of subfertility in clinically indicated individuals. (32)

Fetal investigations are commonly carried out in recurrent miscarriages. These investigations could be invasive or non-invasive. Invasive investigations are chorionic villi sampling (CVS), Amniocentesis and cordocentesis, which are used for karyotyping to identify fetal chromosomal abnormalities, especially trisomy (33). Invasive investigations are carried out for clinically indicated situations since these increase the background risk of miscarriage and damage to fetus tissues. Non- Invasive fetal investigations include testing of cell-free fetal DNA in maternal blood, fetal USS to identify fetal structural abnormalities, and biochemical parameters such as HCG and PAPP-A levels in maternal blood to identify or support fetal abnormalities.

Karyotyping of the product of conception is usually the first line of genetic testing in recurrent miscarriage women following the third miscarriage to identify the genetic abnormality that might be the cause of recurrent pregnancy loss(33).

Role of Assisted reproductive technology

ART is defined as all treatments or procedures that include the in vitro handling of both human oocytes and sperm, or embryos, to establish a pregnancy. This includes, but is not limited to, in vitro fertilisation and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy(34). ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or a sperm donor (35). ART is indicated for several causes of subfertility,

such as females with bilateral tubal factor, diminished ovarian reserve, ovulatory dysfunction, unexplained subfertility and males with severe seminal fluid abnormalities. Besides, certain causes of subfertility, recurrent pregnancy loss and recurrent treatment failure need genetic analysis of the couple to identify the cause related to their genetic material and plan future fertility treatment. ART is the only possible fertility treatment option to identify genetic problems.

The role of ART could be mainly divided into two to overcome genetic issues in a sub-fertile couple.

1. Pre-ART assessment of couple with potential genetics-related problems - Expanded carrier screening
2. Genetic analysis of embryo (pre-implantation genetic testing-PGT) during ART procedure to detect abnormalities and select embryos for transfer.

Pre-ART assessment of couples with potential genetics-related problems (Expanded carrier screening)

Couples with fertility issues might have a high risk of genetic abnormalities. Therefore, pre-ART assessment and counselling are vital for optimal outcomes. Couples with or without a known history of the genetic disease should be assessed and provided genetic counselling for the needed couple by a genetic and fertility specialist before embarking on the ART treatment.

Assessment of the couple includes history, examination and relevant genetic investigations as mentioned above in the assessment of the couple. Expanded carrier screening is the main part of the Pre ART assessment, and it is expanded to include a larger number of genes and variants to identify the possibility of genetic abnormalities to identify the possibility of a child with a recessive genetic disorder after an ART. It is offered to all couples regardless of their ethnicity or region (36).

Fertility specialists and geneticists offer various options to the couple who have highly suggestive genetic issues in their pre-ART assessment such as genetic counselling, pre-implantation genetic testing (PGT) during the ART procedure and third-party ART treatment.

Genetic counselling plays a pivotal role in couples with genetic issues undergoing ART treatment. In which the following points should be discussed with the couples,

- Expected results of the procedure such as failure of the procedure, miscarriage, and genetically abnormal child even after the genetic testing since it only detects the feasible genetic causes (37).
- Couples must be informed that the testing does not guarantee a normal gestation. Therefore, the pregnancy should be followed up with non-invasive or invasive prenatal genetic tests in cases of suspected genetic anomalies since the genetic tests are not 100% accurate (37).
- Explain the procedures and investigation modalities such as chromosomal study.
- Possible complications of the procedure.
- Provide possible solutions with the legal consideration of the respective county with third-party gametes for ART treatment. Third-party ART, defined as donor-assisted conception, includes gamete (sperm or oocyte) and embryo donation for heterosexual couples who cannot conceive with their own gametes or for same-sex couples and single women who want to achieve parenthood(38). however, unresolved genetic problems in the couple also could solve with third-party ART treatment after proper counselling.

After understanding the magnitude of the risk, they should undergo preconception testing.

Genetic analysis of embryo (pre-implantation genetic testing-PGT) during ART procedure

During the ART procedure, the embryo could be checked for feasible genetic problems by pre-implantation genetic testing (PGT) following the embryo biopsy. This might aid to identify the possible genetic causes behind certain causes of subfertility, recurrent pregnancy loss and recurrent treatment failure especially carrying the recessive traits. Embryo biopsy is associated with embryo damage and implantation failure(39).

PGT is defined as a test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA typing or for determining genetic abnormalities. This includes PGT for aneuploidy (PGT-A), PGT for monogenic/single gene defects (PGT-M) and PGT for chromosomal structural rearrangements (PGT-SR) (40).

Commonly, PGT-A is indicated for advanced maternal age, recurrent implantation failure, previous history of genetically affected offspring, family history of genetically affected offspring and recurrent pregnancy loss to detect chromosomal abnormalities. PGT-M is usually carried out to detect monogenic disorders, X-linked, autosomal dominantly and autosomal recessively inherited diseases. Moreover, it might help to detect germline genetic variants which likely cause diseases at birth, in childhood or in adulthood. PGT-SR is mostly indicated in subfertility couples, high risk of pregnancy loss and abnormal live-born babies at birth, usually resulting from the inheritance of unbalanced products of the rearrangement as a routine indication in most ART centres. Meantime, it is only recommended if the technique applied can detect all expected unbalanced forms of the chromosomal rearrangement(38).

Patient understanding and challenges about assisted reproduction

Assisted reproduction is the possible solution for a couple with fertility problems and genetic issues. However, it has several challenges and limitations. Firstly, patient attitude towards ART is good, but the knowledge among people throughout the world seems to be low (35,41,42). Moreover, the public's lack of knowledge of the procedure and outcome of ART may result in couple dissatisfaction since the current success is around 40% after a single cycle. Even though the attitude is positive, couples still have problems with accepting third-party donor gametes due to moral reasons and cultural and religious constraints (43). Apart from that, the high cost of ART and the limited availability of ART centres with genetic analysis facilities are other challenges, especially in low or middle-income countries.

Conclusion

Genetics' impact on fertility, retaining fertility and treatment outcomes without obvious causes is a puzzle to most clinicians, which is currently positively addressed by the modern advancement in the study of genetics and technology development. The causes of fertility issues vary from major changes in the number, structure and function to minor changes in nucleotide sequencing of genes. Couples with these above potential issues need proper assessment with history, examination and appropriate investigations to plan individualised management. The advancement in fertility treatments especially assisted reproductive technology along with genetic analysis, gave hope to couples to overcome certain feasible genetic problems. Further exploration of genetic issues on fertility would positively address in future.

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