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REVIEW

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Sperm genetic abnormality testing in recurrent pregnancy loss cases: a narrative review

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Abstract

Background Recurrent pregnancy loss (RPL), which mostly is of unknown etiology (unexplained RPL, uRPL), is defined as three or more consecutive spontaneous abortions. Recurrent pregnancy loss (RPL) is a problem affecting up to 5% of women of childbearing age due to many factors.

Results The underlying cause is complicated, and the etiology of over 50% of RPL patients is unclear. So far, studies on the etiology of RPL have focused on women, and little attention has been paid to the role of sperm in the development and progression of the disease. Many clinical studies have shown that sperm genetic material and embryonic development potential are closely related to pregnancy outcome. The formation and development of sperm, the combination of sperm and oocyte, and the implantation and development of fertilized oocyte are regulated by chromosome and genes. Because the genome of embryo is provided by sperm, the abnormality of sperm chromosome number and structure, sperm DNA integrity, gene mutation, and epigenetic abnormality may lead to RPL.

Conclusions This article reviews the advances in the studies of the role of sperm genetic abnormalities in RPL, hoping to contribute to the prediction, diagnosis, and treatment of RPL in the future.

Keywords Sperm, Genetics, Recurrent pregnancy loss

Background

Recurrent abortion (RA) is a growing problem all over the world. Recurrent spontaneous abortion (RSA) refers to the consecutive occurrence of fetal loss (body weight < 1000 g) happening more than 2 times before 28 gestational weeks with the same sex partner [1]. Recurrent pregnancy loss (RPL), determined as two or more consecutive abortions by some authors, is seen in 1–3% of couples; an underlying cause, however, is found in up to 50%. Recurrent pregnancy losses could bring physical and psychological harms to the patients, as well as a heavy economic burden, and could even lead to family and social problems [2].

The underlying cause is complicated, and the etiology of over 50% of RPL patients is unclear [1]. So far, the known pathogenesis of RPL comprises only 50% of the causes, which include immune factors, endocrine factors, genetic factors, infection factors, metabolic abnormalities, anatomic abnormalities, and other unexplained factors. Embryo is developed by the combination of sperm and oocyte. Sperm provides half of the genomes in the embryo. Sperm abnormalities may negatively affect embryo development. This review presents an overview on the advances in the studies of the role of sperm genetic abnormality in RPL, hoping to give some help with the prediction, diagnosis, and treatment of the disease. Abnormal number and structure of sperm chromosomes and sperm DNA integrity, gene mutations, and epigenetic abnormalities may induce RPL.

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Main text

Sperm chromosome abnormality and RPL

The disease caused by congenital chromosome number or structure abnormality is called chromosome disease or chromosome syndrome [1]. At present, the research on sperm factors of RPL is relatively concentrated and recognized as sperm chromosome abnormality.

Sperm chromosome number abnormality

Chromosome number abnormality includes aneuploidy (trisomy and monosomy), polyploid, and chimera, among which aneuploid (trisomy) is the most common. The relationship between sperm aneuploidy and RPL is one of the hot issues in reproductive medicine and genetics in recent years. It is because the homologous chromosomes or sister chromosomes are not separated during meiosis of spermatogonia, resulting in the abnormal chromosome number of sperm [2]. In male sperm, there are mainly 13, 18, 21, and sex chromosome dimorphism, among which sex chromosome dimorphism is the most common. Meiosis abnormalities are common in severe oligoasthenospermia and high FSH patients, and abnormal testicular microenvironment is easy to induce [2]. Nazari analyzed the semen samples of 140 male partners of RPL patients and 140 normal fertile men (A. et al.) [3]. The results showed that the mean aneuploidy content of 18 chromosomes ($P<0.01$), 13/21 chromosomes ($P=0.02$), and sex chromosomes ($P=0.015$) of RPL patients was higher than that of normal fertile men. According to Kruger's morphological classification standard, the mean aneuploidy content of normal and abnormal groups was higher than that of normal fertile men, and the mean aneuploid content of sex chromosomes is different, which suggests that there are regulatory genes related to sperm morphology in our sex chromosomes. Sperm with these chromosomal abnormalities can achieve normal fertilization and introduce these aberrations into the embryo, which may increase the risk of abortion, stillbirth, or birth defects. The most frequent fetal chromosomal abnormalities involve the autosomes 21, 18, 13, and sex chromosomes X and Y. Aneuploidy or alterations in copy number of these chromosomes, including trisomy 21 (Down syndrome), trisomy 18 (Edwards' syndrome), trisomy 13 (Patau's syndrome), 45, X (Turner's syndrome), and 47, XXY (Klinefelter's syndrome), account for 80% of clinically significant chromosomal abnormalities diagnosed in the prenatal period [4]. The patients with idiopathic infertility based on a comprehensive andrological examinations including medical history, physical examination, semen analysis, karyotype analysis (patients with azoospermia or oligozoospermia), and Y chromosome microdeletions screening were included in this study [4].

Sperm chromosomal structure abnormalities

The abnormality of sperm chromosome structure is the result of chromosome breakage, but it can be reconnected under the action of repair enzymes. According to the location and number of breaks, the number of involved chromosomes, and the way of reconnection, they can be divided into translocation, inversion, Y chromosome microdeletion, and telomere length abnormality, among which translocation is the most common.

It can be balanced or unbalanced interchromosomal translocation. In a multicenter retrospective study by Hamidian, Talebi, Fesahat, Bayat, Mirjalili, Ashrafzadeh, Rajabi, Montazeri, and Babaei [2], karyotype analysis was performed on 20,432 couples who had experienced RPL. The balanced translocation carriers only changed the relative position of the translocation segments on the chromosomes, retained the total number of the original genes, only changed the relative position of the genes on the chromosomes, and had no serious effect on the role of the gene and the development of the individual. However, abnormal chromosomes easily disturb their germ cells during meiosis, which increases the risk of unbalanced chromosome karyotype. Therefore, the embryos are prone to abortion. Either dysfunction of meiosis-related genes may lead to unbalanced chromosome segregation with consequent aneuploidy progenies and impaired male fertility, or, alternatively, it may cause meiotic restitution, a non-reduction meiotic event resulting in the production of unreduced gametes. Micronutrient deficiencies during pregnancy might lead to spontaneous abortion, fetal malformation, growth retardation, placental abruption, increased maternal morbidity, low birth weight (LBW) babies, neonatal hypocalcemia, and increased incidence of autoimmune diseases. According to the collected case reports, Xue, Zhang, Wang, Wang, An, Sun, and Yu [3] proposed that chromosome 4q, 5q, 7q, 9p, or 14q rearrangement occurred in the sperm of RPL patients, resulting in a relatively high frequency of sperm chromosome translocation. Niederberger, C. et al. [5] reported a family-specific translocation t (2; 20) (p24.1; q13.1) associated with RPL. The rate of miscarriages (50%) in pregnancies from male translocation carriers could be explained by unbalanced translocation-bearing spermatozoa found with a frequency of approximately 55% in the entire sperm population of a t(2;20) (p24.1;q13.1) carrier [6]. Tan, Taskin, Albert, and Bedaiwy [7] reported a case of complex translocation of chromosome (2; 4; 14) and normal semen parameters, but the probability of chromosome aneuploidy of embryo was 100%. In conclusion, the location of sperm chromosome breakpoints and the size of genes involved are the key factors of abortion. Chromosome inversion is a kind of balanced translocation, which can be divided

into inter-arm inversion and intra-arm inversion. Among them, arm inversion of chromosome 9 is the most common, with an incidence of 1–3% [8]. McQueen, Zhang, and Robins [9] found that the relaxing genes are located on the short-arm region of chromosome 9. Thus, we speculated that chromosomal abnormalities of embryos could result in abnormal function expression of regulatory genes in early embryonic development, leading to increased IGF-II gene imprinting loss, which might destroy the balance between villus and deciduae, leading to shallow embryo implantation, spontaneous abortion, and embryonic development arrest [10].

Y chromosome microdeletions are one of the main causes of male infertility. AZFa, AZFb, AZFc, and AZFd have been found [11]. Although in recent years a genetic etiology related to Y chromosome microdeletions has become a major cause of infertility in males with spermatogenesis failures, in this study, the varicocele was the clinical cause of seminal abnormalities that could lead to infertility, suggesting that both varicocele and Y chromosome microdeletion etiologies can present, alone or combined, as factors of male infertility. For example, Liang, W. N. [12] found that the clinical outcomes of ICSI for oligozoospermic patients with Y chromosome AZF microdeletion are comparable to those of infertile patients with normal Y chromosomes. Finally, it seems that Y chromosome microdeletions are not associated with RPL and more research is needed; therefore, performing this test in Iranian couples with RPL is not recommended.

Telomere is an essential DNA–protein complex composed of repetitive DNA and binding proteins to protect the chromosomal ends in eukaryotes. Telomeres are the end structures of chromosomes in mammalian cells; they play a pivotal role in maintaining the stability of the chromosome and become shorter with each cell division. Telomere length abnormality plays an important role in many diseases, but its role in the occurrence of RPL is still unclear [13]. Poorang, Abdollahi, Anvar, Tabei, Jahromi, Moein-Vaziri, Gharesi-Fard, Banaei, and Dastgheib [14] measured the relative leukocyte mean telomere length (T/S) of 25 couples who had experienced idiopathic recurrent pregnancy loss (iRPL) and 20 fertile couples and evaluated the correlation between iRPL and telomere length. The researchers found that the T/S of male and female in iRPL group was significantly lower than that in control group ($P<0.05$). Significant negative correlation was found between age and T/S ($P<0.05$). In sperm parameters, semen volume was negatively correlated with T/S ($r=-0.4679$). Sperm DNA fragmentation index (DFI) was positively correlated with telomere length ($r=0.474$). Researchers suggest that shorter telomere length in men or (and) women may be associated with early pregnancy loss. In germ cells, telomeres contribute to meiotic recombination and homologous chromosome pairing. Telomeres have specialized function in maintaining chromosome integrity and in germ cells and are thought to aid in meiotic recombination and pairing of homologous chromosomes. Telomere shortening in mice reduces synapsis and chiasmata and increases embryo fragmentation, cell cycle arrest, apoptosis, spindle dysmorphologies, and chromosome abnormalities. Sperm with these chromosomal abnormalities can achieve normal fertilization and introduce these aberrations into the embryo, which may increase the risk of abortion, stillbirth, or birth defects.

Abnormal sperm DNA integrity and RPL

Integrity of sperm DNA is vital to transmit genetic information during reproduction, and any damage to DNA could result in infertility. In addition to parameters such as sperm number, concentration, motility, and morphology, another important key marker for sperm quality is chromatin integrity which directly affects reproduction procedure such as fertilization, embryo development, and pregnancy outcome [15]. Sperm DNA damage is a novel indicator of male infertility, which may be caused by an abnormal packaging and segregation of chromatin material, oxidative stress, or abnormal cell apoptosis. In recent years, numerous studies have reported a good correlation between DFI and RPL. The relationship between DFI and RPL has become a hot spot, but the conclusion is not clear. Esquerré-Lamare, Walschaerts, Chansel Debordeaux, Moreau, Bretelle, Isus, Karsenty, Monteil, Perrin, and Papaxanthos-Roche [16] included 16 cohort studies through systematic review and meta-analysis, 2969 pairs of recurrent breast cancer. In 11 cohort studies and 1549 couples treated with assisted reproductive technology, sperm DNA damage was significantly associated with the risk of miscarriage after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). However, Dhawan, Kumar, Deka, Malhotra, Dadhwal, Singh, and Dada [17] found that there was no significant difference in DFI between RPL group and control group, which contradicted the theory that sperm DNA damage was one of the related factors of RPL.

From a clinical perspective, sperm DNA damage, including chromatin fragmentation, has been associated with impaired spermatogenesis and infertility and can have negative consequences on the reproductive process, including recurrent pregnancy loss (RPL) [15].

Gene abnormality and RPL

Synaptonemal complex (SC)-related gene SYCP3, MSH4, and dmnt3l are the structural and functional genes in meiosis of spermatogonia [18–21]. When homologous

chromosomes with such divergent sequences pair with each other and cross over during meiosis, the highly conserved mismatch repair system will be frequently triggered, interfere with normal chromosome disjunction, and lead to faulty chromosome segregation and lethal chromosomal rearrangements/deletions. Biallelic mutations in M1AP are a frequent cause of meiotic arrest and severely impaired spermatogenesis leading to male infertility [22, 23].

Dicer enzyme is the key enzyme in the synthesis of miRNA and siRNA. In recent years, the regulation of reproductive function becomes a research hotspot. Telomeres maintain chromosome stability and genome integrity and also play an important role in meiosis which aid in synapsis, homologous recombination, and segregation. The abnormality of Dicer gene may lead to the occurrence of sperm aneuploidy, which greatly increases the risk of RPL.

Epigenetics is closely related to the occurrence of RPL. Epigenetic regulatory mechanisms such as histone modification, noncoding RNA-mediated regulation, chromatin remodeling, and DNA methylation are emerging as important means of fine-tuning gene expression [24]. Harlev A. et al. [25] conducted a retrospective cohort study on semen samples from 20 males of RPL couples, 147 males of no-RPL couples, and 20 males of childbearing age from 2011 to 2012. The methylenetetrahydrofolate reductase (MTHFR) methylation levels in all semen samples were 55% in RPL males, 8% in no-RPL males ($P<0.05$), and 0% in reproductive age males ($P<0.05$), suggesting that hyper methylation of MTHFR gene promoter may increase the risk of RPL. In addition, Carlini, Paoli, Pelloni, Faja, Dal Lago, Lombardo, Lenzi, and Gandini [26] studied the abnormal methylation of imprinted regions related to sperm development to determine their role in early embryo loss of RPL. Analyzed the methylation levels of H19ICR and DLK1-GTL2, MEST (PEG1), ZAC(PLAGL1), and LINE-1. For H19, the methylation level was significantly lower in the abnormal imprinting group than that in the normal imprinting group ($P<0.05$), but no significant difference was found compared with the control group [27]. Although DNA methylation at the promoter/gene bodies is directly/indirectly correlated with gene expression, this is not strictly true during the periods of dramatic loss of DNA methylation, as occurs during early embryo development or primordial germ cells (PGC) formation [28].

Suggestions for the fertility of men with normal sperm

Regular sex

The frequency of sexual intercourse may actually affect sperm quality. Excessive sexual intervals may lead to inactive semen and a decrease in healthy sperm count.

Keep the testicles cool

In order to produce the best sperm, the testicles must maintain a slightly lower temperature than the rest of the body. Some work or bad living habits may cause people to be in a hot environment for a long time, thus increasing the temperature of the testicles and affecting men's fertility.

Exercise regularly

Exercise can not only improve health and mood but also improve testosterone levels and semen quality. However, it should be noted that overexertion can lower testosterone levels, so pay attention to moderate exercise.

Lose weight

Obesity has proved to be related to infertility, and losing weight can improve sperm quality. Losing weight not only is conducive to overall health but also improves fertility.

Balanced diet

A healthy and balanced diet is essential to maintain sperm quality. The recommended diet includes fruits and vegetables, whole wheat, lean meat, fish, and beans. Nutrients such as vitamin C, vitamin D, and zinc are essential for fertility. Vitamin C can improve semen quality, vitamin D can improve testosterone levels, while low zinc levels in the body are related to poor sperm conditions, and low testosterone levels are associated with increased risk of male infertility. If you want to improve your fertility, enough nutrients may help.

Get enough sleep

As we all know, sleep is crucial to health. Lack of sleep can affect fertility and have a negative impact on sperm health. Adequate sleep can improve mood and overall health and improve the quantity and quality of sperm.

Restrict drinking

Excessive drinking may lead to male fertility problems, which will not only lead to men's loss of interest in sex but also reduce testosterone levels and sperm quality and quantity.

Quit smoking

Smoking can affect almost all aspects of men's fertility, from sperm, quantity, and motor ability to have an erection and the ability to maintain an erection.

Understand the side effects of drugs

Some drugs can have a negative impact on men's fertility. If you are not sure whether the drugs used have an impact on fertility, you should consult your doctor before taking them.

Healthy living habits can improve testicular hormone level, sexual desire, sperm count, etc. and improve fertility. If you always want to have children, but you have not succeeded, please do not be discouraged. Effectiveness requires persistence. If none of these methods work, medication is needed for male infertility.

Conclusion

Although the research conclusions mentioned in this article still have certain limitations in terms of replicability and verifiability, the final orientation of these research conclusions is consistent. RPL affects public health and directly compromises the quality of life of hundreds of women, with a detrimental effect on their physical and mental health. Studies have mostly focused on women, and there is a need for studies on the emotional impact of RPL on men. As a half genome provider of embryos, sperm plays an indispensable role in embryo implantation, growth, and development.

Despite a number of studies showing that RPL and sperm DFI have a strong correlation, the potential mechanism of the effects of high sperm fragmentation on RPL remains unclear. Despite normal semen parameters, male partners in couples with RPL or recurrent implantation failure could have underlying genetic abnormalities in sperm DNA that can be identified.

Many studies revealed the positive association of sperm dysfunction in RPL cases; hence, male may be considered for a routine part of the evaluation along with his partner in the near future in order to achieve desirable outcome.

Abbreviations

RA	Recurrent abortion
RSA	Recurrent spontaneous abortion
RPL	Recurrent pregnancy loss
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
SC	Synaptonemal complex
nRNA	Noncoding RNA
MTHFR	Methylenetetrahydrofolate reductase
iRPL	Idiopathic recurrent pregnancy loss
DFI	Sperm DNA fragmentation index

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