Editorial

Male factor in recurrent pregnancy loss

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Abstract Miscarriage word seems to signify that only women are involved. However miscarriage may be the result of a faulty gamete −male or female. Karyotyping should be carried out if there are two or more miscarriages. In vitro fertilization and preimplantation genetic screening may be carried out in these cases. Prenatal diagnosis has a place in recurrent pregnancy losses (RPL) and should be performed when a translocation or other abnormalities are detected. Men should be given therapy in the form of antioxidants and lifestyle changes that improve overall health and sperm function. In case of obese, men they should loose weight. Regular exercise is important. Diet should be rich in fresh fruits and vegetables. They should limit alcohol and quit smoking. Regular vitamins should be taken. Male factor could be an important cause of embryonic mortality and RPL and should be addressed in all these cases. Hence, more in depth knowledge of epigenetics and genetics is required. As sperm aneuploidy and DNA fragmentation have been shown to be responsible in some cases, it is imperative to have reliable tests for diagnosis of sperm aneuploidy and DNA fragmentation.

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Submission: 26–11–2021, **Accepted:** 27–11–2021, **Published:** 29–12–2021

INTRODUCTION

Miscarriage word seems to signify that only women are involved. However, miscarriage may be the result of a faulty gamete − male or female. Incidence of two or more pregnancies losses occur in 5% of pregnancy and three or more in 1%.[1] Most of the investigations and treatment of a pregnancy loss are for the female partner. The male is not investigated, and not even a semen analysis is included. However, the semen parameters may be normal but there could be a genetic defect which cannot be detected just by semen analysis. About 50% of genes come from the male, which plays an important part in not only early embryonic development but also in placental proliferation later on.

Access this article online

Quick Response Code:

Website: www.fertilityscienceresearch.org

DOI: 10.4103/fsr.fsr_46_21

In males structural chromosomal abnormalities, sperm DNA damage, Y chromosome microdeletions, aneuploidy, and epigenetic changes may be responsible for pregnancy losses.

STRUCTURAL CHROMOSOMAL ABNORMALITY

Incidence of structural abnormalities in chromosomes is higher in couples with recurrent pregnancy losses (RPLs) when compared with the overall population. They are diagnosed by karyotyping. Structural abnormality could be a balanced translocation or a Robertsonian translocation or inversions. Balanced translocations were most frequently found in RPL. Tharapel *et al.* in his review of recurrent pregnancy loss found the prevalence to be 2.9%, five times higher than normal with balanced reciprocal translocations having the highest

How to cite this article: Ghumman S. Male factor in recurrent pregnancy loss. Fertil Sci Res 2021;8:105-10.

incidence of 50%, followed by Robertsonian translocations 24%, and sex chromosomal mosaicisms 12% in females. However, these men do have a chance of having healthy babies. Incidence was twice as much in the female partner, women being more responsible for RPL, even with a genetic cause. They suggested that karyotyping should be performed if there are two or more miscarriages. In vitro fertilization (IVF) and preimplantation genetic screening (PGS) may be performed in these cases. Prenatal diagnosis has a place in RPL and should be performed when a translocation or other abnormality (e.g., X-chromosomal mosaicism) is found with natural conception.[2,3]

SPERM DNA FRAGMENTATION

DNA damage of sperm occurs due to incomplete packaging of sperm chromatin especially protamines which is a process occurring in epididymis, as there is no way to repair a damaged sperm in epididymis or ejaculate.[4] Fragmentation of sperm DNA has shown to impact fertilization, embryogenesis, and further growth leading to pregnancy loss. It can also lead to malformations. If not lethal, it could lead to childhood morbidities such as cancer.[3,5] It has been shown that sperm DNA damage not only impairs fertility but also impairs results of ART. However, the reason why this has not been collaborated by some studies is that may be these studies include intracytoplasmic sperm injection (ICSI) where selection of a better sperm and better embryo may eliminate a DNA damaged sperm or resultant embryo.[6]

Sperm DNA damage may occur because of infection, leukocytospermia, high fever, elevated testicular temperature such as in varicocoele, advanced age, poor diet, cigarette smoking, and exposure to environmental and occupational pollutants.

Protective role of oocyte

Oocytes can reverse DNA damage in sperm by different pathways such as direct reversal of damage, single-strand damage repair, base excision repair, nucleotide excision repair, and mismatch repair.[7] The capacity to repair damage is dependent on amount of mutation in an embryo. If the damage is more the capacity may not be enough to undo harm already carried out. This capacity decreases in older women. Hence, maternal age becomes an important factor where DNA damaged sperm is present.[7]

Importance of DNA damage in the sperms

Paternal genome usually takes over on third day after fertilization at the eight-cell stage. These couples may have normal embryo development while undergoing an IVF cycle till eight-cell stage. DNA fragmentation may be responsible for poor blastocyst formation implantation and early embryo development resulting in pregnancy loss.

In a study, sperm motility, viability, and sperm function test scores were significantly better in the control group when compared with the RPL group.[8] There may be head defects due to alteration in sperm chromatin compaction.[9] Abnormal semen parameters are not a must in DNA damage. In a recent study, it was shown that men with normal semen and recurrent pregnancy loss in partner had a much higher incidence of sperm DNA damage, $36.8\% \pm 5$ compared with controls $9.4\% \pm 2.7$.[10,11]

Studies showed a higher rate of spontaneous abortion in low result with hypoosmotic swelling test.[12] In a study conducted by Saxena *et al.*, they found the sperm function test to be a better parameter to predict RPL than sperm morphology. In their study, sperm morphology as an indicator to recurrent abortion had surfaced only in 5.7% of the total cases in the present study. Abnormal sperm function in the study was 83% for nuclear chromatin decondensation, 46% for acrosomal status test, and 43% for hypoosmotic swelling test.[13] Other studies have shown similar results with an increased rate of abnormal sperm morphology in the RPL group when compared with the control group.[8]

Testing for sperm DNA damage

There are many methods of testing the sperm DNA damage. Sperm DNA testing should be carried out in cases of unexplained infertility, exposure to pollutants, recurrent IVF failure, recurrent pregnancy loss, arrested embryo development, poor blastocyst development, older men, men with varicocele, or oligoasthenospermia.

There are four main methods to test:

(1) Sperm chromatin structure assay (SCSA)
(2) Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay (TUNEL)
(3) Comet assay
(4) Sperm chromatin dispersion (halo) test: A quick test is the sperm Halo test; however, its accuracy is low. Routine testing is not recommended in current guidelines.[14]

Any man with more than 25% sperms showing DNA damage may have subfertility or poor reproductive outcomes. A DNA fragmentation index (DFI) of more

than 50% shows very poor sperm DNA integrity. A cutoff value of 26% was identified by Kumar *et al.* where couples were able to conceive but were unable to sustain pregnancy and experienced recurrent pregnancy loss. As these were idiopathic RPL women, the results of this study show that a high DFI (>26%) did not affect fertilization but adversely affects embryogenesis because of activation of the male embryonic genome 3 days postfertilization. DNA damage may lead to increased mutation load in every cell of the embryo if it escapes the oocyte repair mechanism and may result in postimplantation loss.[7] The cutoff is lower than for infertility and the authors stated that this can well be explained by the fact that in high DNA damage, the impact is severe and manifests as infertility but once DNA damage is down to a level where patient can conceive, it manifests as pregnancy failure.[7]

Can DNA damage be reversed?

There are various methods that decrease the DNA damage such as antioxidant intake, varicocelectomy, and lifestyle changes such as quitting smoking. Recently, an alternative proposed for severe sperm DNA damage is retrieval of testicular sperm and ICSI, as the damage usually takes place in epididymis as mentioned earlier, during packaging of protamines in sperm chromatin.[15]

Measuring sperm DNA has not been brought into use for RPL as there are no defined normograms and cutoff values as there is no definitive proof of link between sperm DNA damage and RPL. In addition, there are many methods to measure sperm DNA, all differing in accuracy. Hence, there is a need for a standard test.

Impact of sperm DNA damage on general health of offspring

Sperm DNA damage is linked with autosomal dominant disorders, cancer, increased prenatal morbidity, congenital malformations, and childhood cancer. Epigenetic changes and DNA mutations along with chromosomal aneuploidies have been associated with increasing paternal age.[16]

Y-CHROMOSOME MICRODELETIONS

Microdeletion of the azoospermic factor (AZF) on the Y chromosome has been found in azoospermic men. The incidence of RPL in partners of these men is to the tune of 32.5%.[17] However, there are some studies that have not found a link between Y-chromosome microdeletion and RPL.[18] A study compared infertile men with those of recurrent miscarriage groups and revealed patients with infertility had different deletions (DYF87S) at AZF region from those with miscarriage.[16] The sperm count test results reported that all males of the control group and also male partners of couples with recurrent miscarriage had normal sperm count ($>20 \times 10^3$), whereas among infertile males, 60% cases had an abnormal sperm count (36.6% were oligospermia and 23.4% were azoospermia). Thus showing that the microdeletions in RPL group may not necessarily affect the sperm parameters but does impact the pregnancy outcome.[19]

ANEUPLOIDY TESTING

Aneuploidy is described as chromosomes in a cell being abnormal in number − could be less or more. Sperm aneuploidy signifies that there is abnormal haploid state of sperm. It is due to abnormal meiosis. Usually nondisjunction is the cause of aneuploidy, but anaphase lag and ineffective checkpoint control lead to aneuploidy.[5]

A study showed that men with partners who have RPL have a 2.7 times higher rate of sex chromosome aneuploidy in sperm, 3.3 times greater rate of sperm with chromosomal 13 or 21 aneuploidy, and 6 times greater rate of sperm with chromosomal 18 aneuploidy compared to controls.[20] Although there have been studies that suggest that sperm aneuploidy has a higher incidence in RPL cases, there have been contradictory studies too. The risk of genetic abnormality in offsprings of men with sperm aneuploidy has not been defined.[14,21,22] Aneuploidy in women is more commonly responsible for RPL than in men.

Aneuploidy testing

Aneuploidy testing is performed by fluorescent *in situ* hybridization (FISH) technology. FISH has detected aneuploidy in men with normal semen parameters and estimates the percentage of sperm with chromosomal abnormalities in a sample. Higher percentage of aneuploid sperms leads to a higher risk of RPL. FISH can also detect increased aneuploidy of a single chromosome which suggests balanced translocation. This may be responsible for RPL. PGS is carried out in these cases.[23]

Drawbacks of testing are

(1) It does not test all sperms and it does not reflect on what the whole sample is like.
(2) Not all chromosomes are tested. Usually testing is of 13, 18, 21, X, and Y and the rest 18 remain untested.

(3) It cannot test the sperm which will fertilize as tested sperm cannot be used for intrauterine insemination or ICSI.

Incidence of sperm aneuploidy and its relation with semen parameters

Aneuploidy may show up in 2% to 9% of men on checking chromosomes 13, 15, 18, 21, X, and Y. This may be due to global impact on meiotic division such as environmental factors or abnormal meiotic recombination. Ramasamy *et al.* showed that men with RPL had a greater percentage of sperm aneuploidy within the sex chromosomes and chromosomes 18, 13, and 21 versus general population (1.04% vs. 0.38%; 0.18% vs. 0.03%; 0.26% vs. 0.08%). The aneuploidy rates were found to be as high as 40% even when normal semen parameters were present. Aneuploidy rates when tested in men with abnormal semen parameters of count and motility increased to 62%. Another marker for aneuploidy was abnormal sperm morphology. There was an increase in incidence of aneuploidy from 28% in normal morphology to 57% in abnormal morphology. However, there was no relationship found between sperm DNA fragmentation and aneuploidy. An increase in aneuploidy did not reflect in an increased sperm DNA fragmentation and vice versa. It was their recommendation that IVF with PGS or early fetal DNA testing in maternal blood should be carried out in RPL.[20]

Protocol to be followed if aneuploidy is detected

An IVF with PGS can be performed.[24] In natural conception with aneuploidy, a noninvasive prenatal testing may be performed along with a nuchal translucency test scan. In case abnormal, further testing by chorionic villous sampling or amniocentesis is recommended. Genetic counseling would involve looking for infertility, recurrent pregnancy loss, birth defects, intellectual disability, and apparent genetic disease in other family members and in the couple. It is important that couples are told about the implications of aneuploidy or translocations if detected. They must also be informed about the increased risk of trisomy. The options must be discussed regarding PGS and donor sperm.

Current status of testing for sperm aneuploidy

In 2015, ASRM practice guideline stated that there may be benefit in testing for sperm aneuploidy in cases of RPL although it should not be routinely performed.[25] The hesitancy in suggesting the FISH analysis also stems from the fact that there is a cost factor and that it does not test some important chromosomes which may be responsible for RPL. Sperm aberrations are mostly nonviable. Tests have been carried out in cases where the pregnancy has survived as with trisomy 13, 18, 21, X monosomy, and Klinefelter (XXY-XXXXY). However, it is the nonviable ones which may be responsible for the RPL.[4,26] At present, there is no treatment to cure sperm aneuploidy. How testing helps is in deciding whether PGS should be carried out or not in RPL, as it would help to select out aneuploid sperm.

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS

Many studies have linked polymorphisms in MTHFR reductase activity with recurrent pregnancy loss. There are many different genetic polymorphisms of MTHFR and some variants are associated more with recurrent pregnancy loss. A meta analysis in 2016 found that both maternal and paternal polymorphisms may be responsible.[27,28] However, other studies have shown an inconsistent result; hence, this test is not included routinely in investigations of RPL.

ANNEXIN A5 M2 HAPLOTYPE

A study showed that genetic factor M2 haplotype, comprises four consecutive nucleotide substitutions in the core promoter of the annexin A5 (ANXA5) gene which resulted in reduced expression levels of ANXA5 in placenta.[28] Annexin A5 (ANXA5) helps in repair of syncitiotrophoblasts and prevents thrombophilia-related damage. Paternal affected gene has been associated with RPL specially from 10th to 15th week of gestation.[29]

UBIQUITIN-SPECIFIC PROTEASE (USP26) GENE ALTERATIONS

It has been observed that some genes on X chromosome in men are related to infertility. Ubiquitin-specific protease 26 has been identified as one of these on the X chromosome that has an important role in spermatogenesis. In a study by Asadpor *et al.* in 2013, it was shown that mutation in USP26 gene in males is associated not only with infertility but also with recurrent pregnancy loss.[30] It has been postulated that this gene alteration can lead to sperm DNA damage.

TELOMERE LENGTH

Telomeres maintain chromosomal integrity. Shortening of telomere results in nonreciprocal translocations,

chromosomal instability, deletions, aneuploidy, and DNA damage.[31] Studies have shown a definite relationship between telomere length and RPL.[32]

EPIGENETICS OF SPERM

Epigenetics refer to noncoding areas in the genome which do not alter the basic DNA sequence but play a regulatory role. Methylation, micro-RNA, etc. can cause an epigenome changes. These may lead to DNA damage.[33] Sperm protamine 1 to protamine 2 mRNA ratio has been associated with male infertility and RPL.[34] A single nucleotide polymorphism in micro-RNA may be responsible for RPL.[35] A 2017 study showed this parental micro-RNA polymorphism to be responsible for miscarriage.[36] Differential DNA methylation profiles are associated with male infertility and RPL.

Although many tests have been suggested but currently only a karyotyping in male has been recognized as an established test in RPL.

INTERVENTIONS FOR THE MALE PARTNER

The role of men in RPL still needs more proof; however, it is recommended that men should be given therapy in the form of antioxidants and lifestyle changes that improve overall health and sperm function. In case of obese men, they should loose weight. Regular exercise is important. Diet should be rich in fresh fruits and vegetables. They should limit alcohol and quit smoking. Regular vitamins should be taken.

It is often reported that couples are stressed because of RPL. Men often tend to take the blame on themselves and get depressed seeing the partner undergoing physical discomfort. However, although emotional concerns are addressed with women, the men remain neglected. It is important to counsel men and ensure that they are emotionally not undergoing a difficult period in a manner similar to women. Men are grieving in this process as well, and everyone needs to remember that. Both the men and the women in a RPL couple need to focus on their self-care.

It is recommended that sperm DNA damage, chromosomal structural abnormalities, and aneuploidy should be assessed. They may be a contributing factor to poor embryo morphology, development, and ultimately failure of pregnancy to progress. In case, sperm aneuploidy is detected; the patient needs genetic counseling as exact risk cannot be assessed. They should be told that there is an increased risk of genetic abnormalities and informed about reproductive options such as PGS, chorionic villous biopsy, donor sperm, or adoption. Males with increased DFI in sperm have the option of going in for testicular sperm and ICSI. Appropriate prenatal tests need to be carried out as follow-up during pregnancies conceived from male partners with increased aneuploidy. A testicular sperm retrieval with ICSI may be attempted where high DNA damage in sperm is present, resistant to any therapy. IVF with PGS to choose normal embryos may be suggested to men with increased sperm aneuploidy and RPL.

Male factor could be an important cause of embryonic mortality and RPL and should be addressed in all these cases. Hence, more in depth knowledge of epigenetics and genetics is required. As sperm aneuploidy and DNA fragmentation have been shown to be responsible in some cases, it is imperative to have reliable tests for diagnosis of sperm aneuploidy and DNA fragmentation.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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