RECURRENT SPONTANEOUS ABORTION AND MALE FACTORS: AN OVERVIEW

Syed Nazar Imam¹, Sami Awda Algaidi², Zarin Rahman³

¹Assistant Professor, Department of Anatomy, College of Medicine, Taibah University, Saudi Arabia.

²Associate Professor, Department of Anatomy, College of Medicine, Taibah University, Saudi Arabia.

³Professor, Department of Obstetrics & Gynaecology, National Medical College, Birganj, Nepal.

ABSTRACT

Recurrent miscarriage is three or more consecutive pregnancy loss before 20 weeks of gestation. Despite detailed investigation of couples experiencing RSA in 40 to 50% of cases, the aetiology cannot be ascertained and are termed Idiopathic Recurrent Spontaneous Abortions (iRSA).

DISCUSSION

In the clinical investigation of male partner of iRSA, semen analysis detailing the sperm parameters do not always reveal significant information that could assist the clinician in patient's treatment. Due to limited predictive potential of semen analysis, new markers with better diagnostic and prognostic characteristic and ability to efficiently predict adverse reproductive events are being investigated. The findings of these studies have reported oxidative stress associated with sperm DNA damage as a leading cause for lower conception rate after assisted conception techniques. These findings have also provided a new direction to the studies investigating molecular markers as free radicals, antioxidants and sperm DNA fragmentation. It has been suggested that these markers are more reliable than semen analysis in male infertility cases including male partners of couples experiencing iRSA. Further, these recent studies also suggest that oxidative stress and sperm DNA integrity may offer better potential to predict sperm reproductive capacity.

CONCLUSION

This review provides an in-depth understanding of iRSA, its causative factors, the clinical findings and the most preferred therapeutics that could assist to improve the pregnancy outcome in such cases.

KEYWORDS

Infertility, Recurrent Spontaneous Abortions, Sperm DNA Damage, Free Radicals, Antioxidants.

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INTRODUCTION

Half of the genetic material is contributed by male gamete and also plays a vital role in embryonic development. Genetic aberration in the sperm may therefore have important consequences on embryonic development and reproductive outcome. Till date semen analysis is the most widely relied tool to assess male fertility,¹ but it is the modest predictor of fertility potential and reproductive outcome.^(2,3,4) Due to limited predictive potential of conventional semen analysis, new laboratory investigations are needed with better diagnostic values that can predict the adverse pregnancy outcome. New studies have revealed that tests for DNA fragmentation may be a better measure.^(2,5) The study of DNA fragmentation is the utmost important in era where advanced forms of assisted reproductive technologies are commonly used. Fertilisation of gametes and subsequent development depends on the integrity of DNA.^(6,7) Recent studies have shown that there must be a threshold of sperm DNA fragmentation beyond, in which embryo development and pregnancy are impaired.^(8,9,10,11) It is now evidential that fragmented human sperm DNA may adversely affect reproductive potential of fertile men.⁽¹²⁾

It is also reported reduced functional competence of sperm with DNA fragmentation and reported a 3-fold increase in abortion rate in cases with high DNA fragmentation.^(9,13-15) Risk for Recurrent Spontaneous Abortion (RSA) increases with age and parity, a woman of old reproductive age group is at greater risk of pregnancy loss than a younger age group women. The incidence of recurrent spontaneous abortion is approximately 1 in 300 pregnancies. However, epidemiologic studies have revealed that 1% to 2% of women experience recurrent spontaneous abortions.⁽¹⁶⁾ About 15% recognised pregnancies resulting in spontaneous loss; there are many more pregnancies that fail prior to being detected. According to one study, only 30% of all conception result in a live birth.⁽¹⁷⁾

Causes of Recurrent Spontaneous Abortion

There are several causes of RSA, important among them being anatomical uterine defects (Congenital uterine anomalies, intrauterine adhesions, uterine fibroids or polyps and cervical incompetence), genetic factors, infections, immunological and environmental factors and blood dyscrasias.⁽¹⁸⁻²⁰⁾ But despite extensive investigation of the female partner, a large number of cases (40-50%) remain idiopathic.^(21,22) It is possible that a number of such cases harbour sperm abnormalities. Till date evaluation of male factors in RSA only involves paternal chromosomal analysis and the role of sperm factors in RSA is totally ignored. With advent of advanced assisted micromanipulation procedures, the role of sperm factors is being realised. Till date about 40-60% RSA cases are identified as idiopathic.

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Corresponding Author:

Dr. Zarin Rahman,

Department of Obstetrics & Gynaecology,

National Medical College,

Birganj, Nepal.

E-mail: dr_zarin@yahoo.co.in

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Definition

Recurrent Spontaneous Abortion (RSA), defined as 3 or more clinical pregnancy losses before the foetus has reached viability.⁽²³⁾

There is general consensus that a diagnosis of RSA requires at least three consecutive miscarriages.⁽²⁴⁾

Incidence

It is a frustrating condition that affects 1% of couples of childbearing age.⁽²⁵⁾ These values vary not only according to the population studied and the means of diagnosing the miscarriage, but also to the age and parity of the patient (4% at 20 years of age versus 16% after 35 years). These differences are greater if biochemical pregnancies are taken into account.

There are several causes of RSA, but despite detailed investigation 40 to 50% cases are idiopathic. Some of the main causes of RSA are discussed below.

Anatomical Causes

These include congenital uterine anomalies, intrauterine adhesions, uterine fibroids or polyps and cervical incompetence.

Genetic Causes

Approximately, 2-4% of all cases of RSA are attributed to genetic causes associated with paternal chromosomal rearrangement like balanced reciprocal translocation (50%), Robertsonian translocation (24%) and inversions, insertions and mosaicism. Several couples also harbour single gene defects.

Infectious Causes

Certain infections like *Listeria monocytogenes*, *Toxoplasma gondii*, Rubella, Herpes Simplex Virus (HSV), Measles, Cytomegalovirus and Coxsackie viruses can cause RPL. There incidence is 0.5% to 5%.

Endocrine Causes

Luteal Phase Defect (LPD), Polycystic Ovarian Syndrome (PCOS), uncontrolled diabetes mellitus, hypo- or hyperthyroidism and hyperprolactinemia are among the common causes of RSA. About 8-12% of total cases of RPL are caused due to endocrinal disorder.⁽²⁶⁾

Immunological Causes

One specific autoimmune disorder, Antiphospholipid Syndrome (APS) is associated with poor obstetric outcome including RSA.

THROMBOTIC CAUSES

Environmental Causes

Three particular environmental and lifestyle factors like smoking, alcohol and caffeine intake have gained particular attention and merit special consideration given their widespread use and modifiable nature. These factors are associated with elevated ROS levels. Radiation can also cause pregnancy loss; radiation above 5 rad is unsafe in pregnancy.

Unexplained Causes

In about 40-50% cases of RSA, no cause is identified and such cases are known as idiopathic miscarriage.

Sperm DNA and its Compaction

Sperm DNA is highly compact due to replacement of about 85% of histone by protamine.⁽²⁷⁾ This is to protect the sperm genome from external stress and degeneration by nucleases.⁽²⁸⁾ The 15% histone are still found at specific DNA sequence and at periphery in association with telomere^(29,30) and has less degree of compaction and may be involved in early human development. Infertile men are found to have increased histone ratio,⁽³¹⁾ which causes poor compaction and make the sperm susceptible to external stress.

Mitochondrial DNA Nucleotide Changes and Pregnancy Outcome

Dysfunctional mtDNA produce lower levels of ATP and higher levels of free radicals resulting in oxidative stress and oxidative stress is the chief cause of nuclear DNA damage. Paternal transmission of mitochondrial DNA is also reported, but these are degraded by 8 cell stage.⁽³²⁾ Kumar et al reported, oxidative stress increases mitochondrial nucleotide alterations and increased nuclear DNA damage. Such dysfunctional mitochondria results in hypospermatogenesis and production of sperm with impaired motility. This may be due to lower ATP levels and disorganised and partially formed microtubular apparatus.⁽³³⁾

CAUSES OF SPERM DNA DAMAGE

Intrinsic Factors/Protamine Deficiency

Protamine is a highly basic protein, which neutralises the negative charge of the DNA phosphodiester backbone and thus packages DNA into a crystalline compact structure, the size of which is 1/6 - 1/20 that of any somatic cell genome.

An important subset of infertile men (about 5%-15%), possess complete protamine deficiency. The defective spermiogenesis could be due to DNA fragmentation and protamine deficiency.⁽³⁴⁾

ROS, Oxidative Stress and Sperm Function

Reactive oxygen species are free radical having one or more unpaired electrons. The common free radical that play an important role in reproduction are superoxide, hydrogen peroxide, peroxy and products of nitrogen.

The reproductive oxidative stress is due to imbalance of ROS production and its neutralisation by antioxidants. The major antioxidants that play an important role of neutralisation of ROS in the seminal fluid are glutathione, superoxide dismutase, reductase and peroxide.⁽³⁵⁾ A shift toward pro-oxidant may be due to more ROS production or low antioxidant are classified as oxidative stress.

Genital tract infection causes increase in leukocytes and level of ROS and subsequently initiates DNA damage.⁽³⁶⁾ Other infections like tuberculosis and pyrexia of unknown origin is also known to have increased ROS and subsequent initiation of DNA damage.

Febrile illness, certain lifestyle factors are associated with testicular hyperthermia which reported to cause increase in histone and protamine ratio and initiation of sperm DNA damage.⁽³⁷⁻³⁹⁾

It is found in the follicle stimulating hormone receptor knockout mice that low testosterone level is associated with high level of DNA damage.⁽⁴⁰⁾

Abortive Apoptosis

Abortive apoptosis may result in sperm DNA damage, but this theory was previously challenged. It causes destruction of most of the spermatozoa during normal spermatogenesis. Sakkas et al reported that spermatozoa with damaged DNA have escaped apoptosis because of dysfunctional mitochondria. Germ cell apoptosis is found to be disrupted in people with old age and men with treatment on anticancer therapy.⁽⁴⁰⁻⁴³⁾

Extrinsic Factors

Cancer and cancer treatment, both chemo- and radiotherapy are associated with DNA damage and poor semen quality⁽⁴⁴⁻⁴⁷⁾ and are strongly advised to have their sperm cryopreserved.⁽⁴⁸⁾

Men with varicoceles are found to have high level of oxidative stress and associated damage of DNA⁽⁴⁹⁾; recently it is found that men with varicoceles there is abnormal retention of sperm cytoplasmic droplets and subsequent DNA damage.^(50,51)

It has been reported by some studies that ROS level decreases rapidly in 1 week post-surgery, but significant sperm DNA integrity occurs only after 5-6 months post-surgery.^(52,53)

Cigarette smoking: Abnormal semen parameters and DNA damage is reported in men who smoke regularly is probably due to testicular inflammation and activation of leukocytes leading to increased production of leukocyte derived ROS⁽⁵⁴⁾ and its level is significantly increased in these men.⁽⁵⁵⁾

Factors which can Decrease Sperm DNA Damage

Lifestyle modification like quitting smoking, alcohol, minimising exposure to high temperature (Sauna, hot bath) and exposure to various pollutants, exercising in moderation, yoga, intake of fruits and vegetables can decrease oxidative stress and thus result in decrease in DNA damage.⁽³⁶⁻³⁹⁾

As local and systemic infection can cause increase in ROS levels and thereby causes significant DNA damage, such cases with high ROS level benefit immensely by treatment of infection, anti-inflammatory agents and antioxidant supplements. Recent studies have shown that vitamin C increases sperm concentration and vitamin E increases motility, but antioxidants should only be given to men with increased ROS levels since physiological ROS levels serve many important functions.⁽³⁵⁾ Varicocele is associated with high ROS levels and DNA damage and such cases benefit immensely by varicocelectomy.⁽⁵⁰⁾ Antioxidant can also be used in vitro in culture media in ART to prevent ROS mediated damage during spermiogenesis.

Thus there are several causes of RSA, but to understand if paternal factors play a causal role in a large fraction of cases identified as idiopathic is the aim of this study.

DISCUSSION

Recurrent spontaneous abortion is three or more consecutive pregnancy loss before 20 weeks of gestation.⁽⁵⁶⁾ Although some causes have been identified, still in 50% of couples the underlying mechanism (s) are not identified. It is possible that in large percentage of idiopathic cases, there is an underlying male factor which has been ignored and is the aetiology for recurrent pregnancy loss. These male factors are rarely studied or analysed in RSA.

Some studies have reported that sperm DNA damage may be associated with compromise of early embryonic development.^(57,58) In case of RSA, the father is merely investigated for chromosomal trisomies in most of the centre and infertility and RSA are mainly considered to be due to an underlying female aetiology. Though chromosomal aberrations are the causal factor in 2-4% of cases experiencing RSA, approximately 2-4% of all cases of RSA are attributed to genetic causes associated with parental chromosomal rearrangement like balanced reciprocal translocation (50%), Robertsonian translocation (24%) and chromosomal inversions, insertions and mosaicism. It is emphasised that a number of cases may also have non-specific DNA damage and which cannot be detected by simple cytogenetic analysis and such DNA damage may result from oxidative stress.⁽⁵⁹⁾

Oxidative stress is found to be one of the chief causes of DNA damage, which leads to sperm dysfunction and occurs due to imbalance between oxidant levels and antioxidant defence mechanism.

Sperm are highly vulnerable to oxidative stress as spermatozoal membrane is found to have high content of polyunsaturated fatty acid and low cytosolic antioxidants, as majority of cytoplasm is lost during spermiogenesis. Our body has both enzymatic and non-enzymatic antioxidants. Low levels of antioxidants and excess free radicals damage all biomolecules and reduce sperm functional competence. This is a protective mechanism, as such sperm are unable to fertilise the ova; however, if they are successful in fertilisation it may result in pre- or post-implantation losses.

A previous study⁽¹⁴⁾ documented that infertile men with normal or abnormal sperm parameters have raised ROS levels and decreased antioxidant levels and high levels of nuclear DNA damage. However, it is difficult to predict increased ROS levels and DNA damage based on standard semen parameters. Thus, tests for seminal oxidative stress and DNA integrity are necessary to understand the reproductive potential of the sperm. Therefore, the attention has now shifted from analysing standard semen parameters to studying/evaluating molecular aspects of spermatozoa. Among these tests evaluating sperm DNA damage, free radical estimation, sperm transcript levels and telomere length are the focus of recent studies.

Oxidative stress and DNA damage is found in infertile men with normal or abnormal semen parameters⁽¹⁴⁾ and so it is difficult to predict the DNA damage on the basis of standard semen parameters. Thus, tests for sperm oxidative stress and DNA damage are important to evaluate the sperm reproductive capacity. So the shift is from standard semen parameter to comprehensive evaluation of molecular aspect of sperm; among these tests are evaluating sperm DNA damage, estimation of free radicals and telomere length are focus of recent studies.

Polyunsaturated fatty of spermatozoa are highly vulnerable to ROS attack causing defective membrane permeability, ATP losses and subsequently sperm motility and morphology defects.^(60,61) So this Lipid Peroxidation (LPO) apart from sperm membrane damage also causes DNA damage leading to infertility.⁽⁶²⁾

Sperm DNA damage detected in infertile men are found to be associated with high ROS.⁽⁶³⁾ As observed in a pilot study that individuals with DFI ≥ 16.5 have significantly impaired motility and morphology.⁽⁵⁷⁾ Dada et al reported that raised

ROS levels lead to both mitochondrial sequence variation and nuclear DNA damage.⁽⁶¹⁾ Mitochondrial sequence variation result in dysfunction of mitochondria, which generates less ATP and impairs motility, hypospermatogenesis and may also be the underlying pathology in infertility and RSA.⁽²⁾ Since OS is an important factor for sperm DNA damage, it is postulated that it also induces sequence variation in mitochondria and thereby reducing ATP production, increased free radical generation and impairs motility of sperm and this is primary effect of mitochondrial dysfunction.

Now, it is established that sperm DNA fragmentation are associated with RPL and male infertility, but it is yet to be established that what is the threshold for DNA Fragmentation Index (DFI) which is associated with these effects of sperm DNA damage because the DFI threshold value of 16.50%⁽⁵⁷⁾ is found in the study in men of couple experiencing RPL. Another study reported low sperm DNA fragmentation (12%) in the group that resulted in pregnancy than those that did not⁽⁶⁴⁾; but to further validate the threshold value from these studies large sample size is required. Negative correlation of sperm DNA fragmentation with fertilisation and embryo cleavage rate was reported.⁽⁶⁵⁾ However, no association of DNA fragmentation on IVF and ICSI outcome was found by TUNEL assay, but they observed high fertilisation rate in group with low DNA fragmentation <10%. Recent study by Dada et al reported that infertile men with repeated ICSI failure had increased sperm DNA fragmentation. Various studies documented SCSA as a reliable method for studying sperm chromatin integrity. Studies have reported threshold cut-off values of DFI between 20%-30% in infertile men opting for ART,⁽⁶⁶⁻⁶⁹⁾ but in cases of iRSA the cut-off values by various methods of sperm DNA fragmentation assay have not been reported and the cut-off values also vary with the methods used for the sperm DNA integrity assessment and lacks standardised laboratory protocols. It has been reported that there is a 3-fold increase in miscarriage rate in cases with high DNA fragmentation. This may be a mechanism of natural selection, whereby embryos with intact DNA integrity could only complete development. Recent studies have shown that sperm DNA damage correlates with infertility, early pregnancy loss, defective embryogenesis, congenital malformations, genetic abnormalities and decreased fecundity and DNA sperm methylation pattern.⁽⁷⁰⁾

Till date majority of studies have focused on role of chromosomal abnormalities and analysis of nuclear genes for nucleotide alterations, which affect embryonic development. This study analyse role of oxidative stress, DNA damage and mitochondrial DNA variation in iRSA following spontaneous conception.

The role of OS and DNA damage in iRSA is an area deserving intense research. This is a preliminary genetic study which included analysis of sperm DNA damage, assessment of whole mtDNA sequence variation and assessment of ROS levels, which should be followed up by larger studies in different ethnic populations worldwide. This study aims to understand the role of ROS and nucleotide alterations in cases. Thus, a comprehensive genetic diagnostic workup in male partners is important in iRSA to understand exact aetiology, provide counselling and most adapted therapeutics to patients.

CONCLUSION

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included analysis of sperm DNA damage, assessment of whole mtDNA sequence variation and assessment of ROS levels, which should be followed up by larger studies in different ethnic populations worldwide. This study aims to understand the role of ROS and nucleotide alterations in cases. Thus, a comprehensive genetic diagnostic workup in male partners is important in iRSA to understand exact aetiology, provide counselling and most adapted therapeutics to patients.

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