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Review Article

The Role of Genomic Instability in Unexplained Infertility: An Overview

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Abstract

Unexplained infertility (UI) is an ambiguous condition that afflicts a significant number of couples around the world, despite normal findings in routine infertility tests. This review examines the role of DNA damage, especially in spermatozoa, as a potential cause of unexplained infertility. Sperm DNA fragmentation (SDF), often not detected in conventional sperm analysis, has emerged as a key biomarker for assessing male fertility potential. Elevated DNA fragmentation index (DNA) levels, caused primarily by oxidative stress, environmental toxins and incorrect chromatin remodelling, have been associated with poor reproductive performance, including miscarriage and Assisted Reproductive Technologies (ART). In addition, subclinical factors in women, such as early-stage endometriosis or luteal phase defects, may contribute to the diagnosis of uterine fibrosis. Advanced diagnostic techniques such as Terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL), Sperm Chromatin Structure Assay (SCSA) and Comet Assay offer more accurate detection of DNA damage. An understanding of these molecular factors is essential for specific treatment strategies and to improve the success rate of both natural and artificial insemination.

Keywords: unexplained infertility, DNA damage, sperm DNA fragmentation, oxidative stress, DNA fragmentation Index, assisted reproductive technologies

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1. Introduction

Infertility is the condition where a couple cannot conceive or get pregnant after 12 months of unprotected intercourse [1]. The idea of unexplained infertility cases was proposed in the 1960s [2]. Infertility can be further classified as Primary, Secondary and Unexplained infertility. Causes, including ovulatory disorders such as Polycystic Ovary Syndrome (PCOS), low sperm count and hormonal disorders, can contribute to infertility. In males, several studies have shown a declining semen quality and various sperm abnormalities as a cause for infertility, whereas in females, they are due to hormonal imbalance and disorders such as PCOS, along with others [1]. 30% to 40% of the total infertility cases are seen in men. UI accounts for almost 40% of female infertility and 8-28% of infertility in couples [3].

1.1. DNA Damage

DNA damage is defined as any or all modifications in the physical or chemical structure of DNA that result in a DNA molecule that is different from the original form with respect to its chemical or physical properties. Substances that cause DNA damage are called genotoxic agents.

Genotoxic agents can cause direct DNA damage as well as induce changes in the gene, which could lead to further DNA damage [4].

1.1.1. Causes of DNA damage

DNA damage can result from both exogenous and endogenous factors. Exogenous factors include environmental agents such as ionising radiation, chemicals and ultraviolet light, whereas endogenous factors arise from normal cellular metabolic processes. Interestingly, endogenous sources are responsible for DNA damage more frequently than exogenous ones. Despite their different origin, both types of factors can induce genotoxicity through similar mechanisms. For instance, ionising radiation (an exogenous factor) and oxidative phosphorylation (an endogenous process) both lead to the generation of reactive oxygen species (ROS). When produced in excess, ROS can cause significant damage to the DNA. Genotoxic agents may be physical, such as UV and IR, or chemical, such as alkylating and oxidizing agents. These agents can inflict damage by inducing DNA-DNA or DNA–protein crosslinks, strand breaks, or base modifications. Whether physical or chemical, exogenous or endogenous, all these agents contribute to genomic instability through overlapping and often synergistic mechanisms [4, 5].

1.1.2. Types of DNA damage

DNA Damage can occur in various forms, affecting genome stability and cellular function. Mismatched bases arise from base modifications like deamination (eg. Cytosine to Uracil) or methylation, leading to

incorrect base pairing and potential protein alterations or premature stop codons. Single-strand breaks (SSBs), the most common type of DNA damage, result from phosphodiester bond disruption during transcription, replication, or oxidative stress and are usually repaired efficiently [4, 6]. Double-strand breaks (DSBs) involve breaks in both DNA strands and may arise from clustered SSBs; they can lead to chromosomal aberrations if unrepaired, although they also occur naturally during meiosis and immune cell differentiation [4, 7]. UV radiation induces dimer formation, such as cyclobutane pyrimidine dimers and 6-4 photoproducts, which distort the DNA helix and trigger cell cycle arrest or apoptosis. Bulky adducts, formed by carcinogenic chemicals like polycyclic aromatic hydrocarbons and industrial solvents, covalently bind to DNA, obstructing replication and transcription, often resulting in cell death if not repaired [4].

1.2. Infertility in Females and Males

Unexplained infertility (UI) remains a multifactorial condition with both female and male contributing factors. Among female-related causes, endometriosis has been widely hypothesised as a predominant factor. It is identified in approximately 30–50% of subfertile women and nearly 5% of fertile women. Laparoscopic evaluations of women diagnosed with UI have reported endometriosis in approximately 15% of cases. Furthermore, randomized studies have demonstrated improved natural conception rates following the surgical excision of endometrial lesions. Uterine anomalies, such as fibroids, have also been implicated in impaired fertility outcomes [8].

Male factors are increasingly recognized as important contributors to UI. Sperm DNA integrity is essential for successful fertilization and the development of healthy offspring. While DNA imperfections are present in all men, they are generally observed at higher levels in infertile and subfertile individuals [9-11]

Elevated sperm DNA fragmentation (SDF) is associated with reduced reproductive success, both in natural conception and assisted reproductive techniques, including in vitro fertilization (IVF) and intrauterine insemination (IUI). A study investigating the role of male factors in UI reported that 46% of male partners exhibited a DNA fragmentation index (DFI) greater than 15%, despite having normal semen parameters, indicating a significant prevalence of sperm DNA damage [9]. Oxidative stress represents a principal mechanism underlying sperm DNA fragmentation, triggered by both endogenous and exogenous factors, several of which are modifiable. Although the impact of sperm DNA damage on male infertility is increasingly recognized, its inclusion in routine semen analysis remains a subject of ongoing debate [12, 13].

The spermatozoa population within an ejaculate is typically heterogeneous. A strong correlation exists between elevated levels of DNA damage and poor sperm parameters, suggesting underlying spermatogenic defects in affected individuals. Gene mutations, environmental stressors, and chromosomal

abnormalities can disrupt chromatin architecture, contributing to DNA damage through impaired chromatin packaging, apoptosis, or oxidative stress [14]. Reactive oxygen species (ROS) are continuously generated in living cells; however, excessive ROS production can overwhelm cellular antioxidant defences, resulting in oxidative stress and subsequent cellular injury. Elevated ROS levels have been consistently observed in males with idiopathic infertility [8], further implicating oxidative stress as a significant mediator of male reproductive dysfunction [15].

Assisted reproductive technologies (ART) have emerged as effective tools for identifying and addressing factors such as sperm and oocyte abnormalities that hinder the formation of embryos or blastocysts during the cleavage stages [Wang]. ART encompasses a range of techniques, including intracytoplasmic sperm injection (ICSI), in vitro fertilization (IVF), gamete or embryo cryopreservation, and the use of fertility medications. When employed to overcome infertility, these interventions are collectively referred to as fertility treatments. ART primarily focuses on the fields of reproductive endocrinology and infertility [1].

Infertility remains a significant public health concern, particularly in South India, with higher prevalence rates reported in states such as Kerala, Tamil Nadu, Karnataka, and Telangana. A study conducted in Kerala among married women aged 20 to 49 years who had been married for at least two years reported an infertility prevalence of 11.1%. Globally, approximately 60 to 80 million couples experience infertility each year, with an estimated 15 to 20 million affected couples residing in India [1]. Within this context, unexplained infertility accounts for approximately 10–20% of all infertility cases among Indian couples [8].

1.2.1. Types of Unexplained Infertility

Unexplained infertility is diagnosed when a couple fails to conceive after one year of regular, unprotected intercourse despite having no identifiable abnormalities on standard infertility evaluations [16,17]

These evaluations typically include assessments of ovulation, tubal patency, and semen parameters. The absence of any discernible cause leads to a diagnosis of unexplained infertility, which affects a significant proportion of infertile couples worldwide [9]. However, emerging research suggests that “unexplained” may often be a misnomer, reflecting limitations in current diagnostic sensitivity rather than a true absence of causative factors.

Based on current understanding, unexplained infertility can be broadly categorized into the following subtypes:

1.2.2. Idiopathic Unexplained Infertility

This classic form of unexplained infertility is characterized by normal results across routine investigations, including hormonal profiles, pelvic imaging, tubal patency assessments, and semen analyses. In these

cases, the infertility remains genuinely unexplained based on available diagnostic modalities, presenting considerable clinical challenges due to the lack of clear therapeutic targets [9].

1.2.3. Male Factor-Related Unexplained Infertility

While conventional semen analysis has long been the cornerstone of male fertility evaluation, it often fails to capture more subtle defects in sperm function. Many men with normal sperm concentration, motility, and morphology may nonetheless exhibit elevated sperm DNA fragmentation (SDF), a condition associated with impaired fertilization, compromised embryo development, and increased miscarriage rates [9,14]. In a recent study of 200 couples with unexplained infertility, approximately 46% of male partners demonstrated elevated SDF despite normal semen parameters [9]. These findings emphasize the importance of assessing chromatin integrity as part of the diagnostic workup for unexplained infertility [18].

1.2.4. Subclinical or Occult Female Factors

Some cases of unexplained infertility may be attributable to subtle or early-stage reproductive pathologies that evade detection by conventional assessments. These may include:

- Minimal or early-stage endometriosis not visualized through standard imaging,
- Luteal phase defects or subtle ovulatory dysfunction,
- Mild thyroid or metabolic disturbances falling within borderline thresholds,
- Mild tubal damage or peritoneal adhesions impairing gamete or embryo transport without causing complete tubal obstruction.

Such factors are often referred to as “occult” or “subclinical” contributors and may only be identified through more advanced or invasive diagnostic techniques [1].

In certain cases, infertility may arise from the cumulative effect of minor abnormalities in both partners. For example, a female partner with subtle ovulatory dysfunction and a male partner with moderate SDF may, in combination, experience significantly reduced fertility potential, even though neither individual shows overt pathology when assessed separately. This multifactorial interplay highlights the importance of evaluating infertility at the level of the couple as a functional biological unit rather than isolating investigations to each partner independently [1].

1.3. Evaluation of DNA Damage

Various assays are employed to assess DNA damage, each providing valuable insights into the extent and nature of DNA fragmentation. The Comet Assay is a widely used technique that quantifies both single- and double-stranded breaks in DNA by observing the characteristic "comet" shape formed when cells are stained with fluorescent dyes, such as SYBR Safe. Increased DNA strand breakage is indicated by a greater fluorescent intensity at the comet tail [Agarwal]. Similarly, the TUNEL Assay detects DNA fragmentation associated with apoptosis, relying on the enzyme terminal deoxynucleotidyl transferase (TdT) to catalyze the binding of deoxyribonucleotides to DNA double-strand breaks, thus identifying excessive DNA damage at the cellular level [14, 19].

The Sperm Chromatin Dispersion Test (SCD) is a simpler method used to assess the DNA Fragmentation Index (DFI), based on the principle that cells with fragmented DNA fail to form a characteristic halo when mixed with agarose following nuclear protein removal and acid denaturation. Another method, the Sperm Chromatin Structure Assay (SCSA), is a cytometric technique that evaluates chromatin abnormalities by measuring a metachromatic shift in fluorescence after acid treatment, with intact double-stranded DNA emitting green fluorescence and denatured single-stranded DNA emitting red fluorescence. Finally, the In Situ Nick Translation (NT) Assay quantifies the incorporation of deoxyuridine triphosphate (dUTP) at single-strand DNA breaks, catalyzed by DNA polymerase I, to identify cells with varying levels of endogenous DNA damage [14, 20].

2. Conclusion

Infertility is a complex disorder influenced by both genetic and external factors. While specific gene mutations have been linked to male infertility, many underlying factors leading to sperm DNA damage remain unknown. DNA damage, particularly due to elevated DNA Fragmentation Index (DFI), is a significant factor in such cases. The presence of genotoxic agents and the heterogeneity of sperm compromise chromatin integrity, and therefore impact fertilisation, embryo development, and overall reproductive success.

Advanced semen analysis, such as TUNEL assay, SCSA, and Comet assay, highlights a deeper layer of dysfunction compared to conventional methods, which may indicate normal parameters. The role of oxidative stress, chromatin remodelling errors, and DNA strand breaks (both single and double) broadens our understanding of male infertility and also explains idiopathic reproductive failure. Furthermore, subclinical or undetectable female factors, such as early-stage endometriosis or luteal phase defects, are major causes of unexplained infertility in females.

Artificial Reproductive Techniques (ART) have been crucial in overcoming some barriers linked to unexplained infertility. However, improving natural conception rates will depend on more tailored,

molecular-level diagnostics and targeted treatment approaches. A comprehensive understanding of DNA damage and repair mechanisms is essential for developing accurate diagnostic tools and individualized treatments in cases of unexplained infertility.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

Aaron M. Thomas, Melita Mary George, Nevil George Ninan, Sandra Maria Antony, Merlyn Prince, and Angela John contributed to the conceptualization and drafting of the manuscript. Adithya R. Pillai contributed to the correction of the manuscript. Anjali Anne Jacob and Riju Mathew contributed to the final review and revision of the draft. All authors read and approved the final version of the manuscript.

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