DNA Fragmentation in Human Spermatozoa: Types, Causes and Method of Detection

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***Abstract*: Sperm DNA integrity is essential for effective fertilization, embryo growth, pregnancy, and genetic material transmission to progeny. DNA fragmentation is the most common DNA abnormality in male gametes, and it has been linked to poor semen quality, low fertilization rates, poor embryo quality, and preimplantation development, as well as poor clinical outcomes in assisted reproduction techniques. This paper outlines thetypes, causes and method of detection of spermatic DNA fragmentation.**

1. Introduction

Approximately 10−15% of couples of reproductive age are unable to conceive within twelve consecutive months of unprotected intercourse and therefore they are characterised as infertile. A male factor has been implicated in almost 50% of the cases, either solely (20%) or in combination with the female factor (30−40%) (Evgeni et.al).

Male infertility is typically measured by the quality of sperm. The most essential criteria tested at infertility centres as part of standard semen analysis are ejaculate volume, sperm concentration, motility, and morphology determined according to the World Health Organization (WHO). The genetic composition of a baby is the outcome of egg and sperm DNA information, and it should be preserved for ongoing embryo and fetal growth and healthy progeny. Damage to the DNA of male or female gametes might cause the reproductive process to be interrupted. Sperm DNA fragmentation, which is observed in a high percentage of spermatozoa from subfertile and infertile males, may be the most common cause of paternal DNA abnormality transfer to offspring(García-Ferreyra et al.).Intrinsic causes such as abortive apoptosis, recombination deficits, protamine imbalances, and oxidative stress can all induce sperm DNA fragmentation. Extrinsic factors such as storage temperatures, extenders, handling circumstances, time after ejaculation, infections, and drug reactions, as well as post-testicular oxidative stress, might cause damage.
Protamination and the lack of DNA repair are two distinct traits that distinguish sperm from somatic cells. As transcription and translation in sperm ceases after spermiogenesis, these cells lack the ability to repair DNA damage sustained during their transit through the epididymis and post-ejaculation. Several studies have found that while DNA fragmented spermatozoa can fertilize an egg, they are associated with poor embryo quality, arrested blastocyst development, and poor pregnancy rates if used naturally or through IUI, IVF, or ICSI techniques. Several studies show that the oocytes and embryos retain the potential to repair DNA damage in the paternal genome; however, it is unclear if all types of damage can be repaired. Sperm have no mechanism for repairing DNA damage that occurs during travel and storage in the epididymis, as well as after ejaculation. Some types of sperm DNA breakage have been demonstrated to be repaired by ocytes and early embryos. As a result, the biological impact of aberrant sperm chromatin structure is determined by the interaction of the quantity and kind of sperm chromatin

2. Objective

To understand the types, detection and mechanisms that cause DNA fragmentation in human sperm, including those that occur during spermatogenesis and during transportation through the reproductive canal. Apoptosis in the seminiferous tubule epithelium, chromatin remodelling defects during spermiogenesis, oxygen radical-induced DNA damage during sperm migration from the seminiferous tubules to the epididymis, activation of sperm caspases and endonucleases, damage caused by chemotherapy and radiotherapy, and the effect of environmental toxicants are among the mechanisms investigated.

3. Human Sperm Chromatin Structure

Spermatogenesis is a continuous and highly managed process that results in a creation of highly specialised sperm cells. Spermatogonial stem cells divide and develop into primary spermatocytes, which go through genetic recombination to produce round haploid spermatids. The round spermatids next go through a process called spermiogenesis, which involves significant cellular, epigenetic, and chromatin remodelling. The nucleosomes and histones are dissolved, and the highly positively charged protamines are replaced, generating tight toroidal complexes that organize 85—95 % of sperm DNA. There are two forms of protamine in human spermatozoa (P1 and P2). Human sperm chromatin is less stable because P2 contains fewer thiol groups for disulfide bonding. Finally, when the cysteines travel through the epididymis, they are gradually oxidised, generating inter- and intraprotamine disulfide connections that, along with zinc bridges, stabilise and compact the chromatin completely. Thus sperm chromatin is a crystalline, well-organized structure made up of haploid DNA and heterogeneous proteins. Its highly condensed and insoluble structure which protects paternal genetic material during transmission through the male and female reproductive systems, adjusting in the extremely small volume of sperm nucleus(Javier García-Ferreyra et.al).The model proposed by Ward and Coffey depicts the organisation of sperm chromatin as long strands of DNA that are gradually packaged at four levels: (1) Chromosomal anchoring, or the attachment of DNA to the nuclear annulus. (2) Formation of DNA loop domains, or the attachment of DNA to the

nuclear matrix.(3) Replacement of Histone by protamines, which condense DNA into compact "doughnut"-shape arrangements.(4) chromosomal organization(EvangeliniEvgeniet.al).

4. Types of DNA damage

Only the two-tailed Comet assay can distinguish between single-strand (SSBs) and double-strand (DSBs) DNA breaks. Increased levels of the phosphorylated isoform of the H2AX histone are linked to DSBs and have been proposed as a DSB molecular biomarker. The etiologies of SSBs and DSBs in sperm DNA, their relationship to reproductive outcomes, and the processes involved in their repair are all reviewed (Agrawal et al.,2020) Evidence suggests that DSBs have a more detrimental influence on reproductive outcomes (fertilization, implantation, miscarriage, pregnancy, and live birth rates) than SSBs, which can be mitigated in part by employing intracytoplasmic sperm injection (ICSI).

There are various types of DNA damage in mammalian germ cells. The male gamete does the majority of the damage. Single (SSB) and double DNA strand breaks (DSBs) describe DNA fragmentation, which is particularly common in the ejaculates of infertile males. Single and double strand DNA breaks can be detected using TUNEL and Comet tests. The development of 8-OH-guanine and 8-OH-20-deoxyguanosine (8-OHdG) and single-stranded DNA fragmentation follow sperm DNA fragmentation caused by oxidative assaults such as the hydroxyl radical and ionizing radiation. Through the activation of sperm caspases and endonucleases, hydroxyl radical production may result in the indirect induction of double-stranded sperm DNA damage (González et al.,2012).

5. Origin of sperm DNA damage

5.1 Intrinsic Cause

5.1.1 Recombination Errors during Spermatogenesis

The structure of sperm chromatin is a complicated arrangement of DNA and sperm nuclear protein with various levels of compaction to reduce the nuclear volume and head size. So, DNA fragmentation could be the result of unresolved strand breaks formed during spermiogenesis to relieve the torsional strains involved in packing a high quantity of DNA into a small sperm head. Thisremodeling process necessitates the transitory creation of physiological strand breaks, spermatidsbeing haploidcell,are unable to repair through homologous recombination. When DNA strand breaks occur in somatic cells, nuclear poly(ADP-ribose) polymerases rapidly produce poly(ADP-ribose), which enables DNA strand break signalling and the building of DNA repair complexes (Mirella L. Meyer-Ficca et.al).

5.1.2 Maturation of aberrant spermatids.

DNA breaks are required for temporary relaxation of torsional stress, allowing the nucleosome histone cores to be come off and replaced by protamines. Endogenous nuclease activity is required to relax the chromatin via histone hyper-acetylation and introduce breaks by topoisomerase II, which is capable of both producing and ligating breaks. During epididymal transit, chromatin packaging around the new protamine cores should be finished, and DNA integrity restored. DNA fragmentation in ejaculated spermatozoa can develop if the transient breaks are not repaired.

5.1.3 Protamine 1 and Protamine 2 ratio.

Male infertility is linked to variations in sperm protamine expression. In humans, 85–95 percent of histones are replaced by sperm during late spermiogenesis in a multi-step process. Histones are first hyperacetylated, then testes-specific variations of histones are replaced, and finally transition proteins are used to replace them(González-Marín, Gosálvez and Roy, 2012).Protamines 1 and 2 are then used to replace the transition proteins (P1, P2). P1 and P2 are generally produced in human sperm in a 1:1 ratio and create a tight packaging of the sperm DNA, leading in nucleus compaction and termination of gene expression. Abnormally high and low P1/P2 ratios,is also responsible for DNA fragmentation(Simon, Castillo, Oliva and Lewis, 2011).

5.1.4 Oxidative Stress

ROS, or free radicals, are oxidising agents produced as a consequence of oxygen metabolism. The chromatin in the sperm nucleus has been shown to be sensitive to oxidative stress, resulting in base alterations and DNA fragmentation. The chromatin in the sperm nucleus has been shown to be sensitive to oxidative stress, resulting in base alterations and DNA fragmentation. Electromagnetic radiation causes ROS generation, which leads to DNA damage and decreased motility and vitality in human spermatozoa(De luliis et al). Furthermore, several toxins released from structural materials or industrial products (e.g., benzene, methylene chloride, hexane, toluene, trichloroethane, styrene, heptane, and phthalates) as well as toxins in the form of metals (e.g., cadmium, chromium, lead, manganese, and mercury) increase ROS production in the testes(Jurasović, J et al.).Smoking and drinking also cause increased formation of reactive oxygen species (ROS) and DNA strand breaks, resulting in a decrease in sperm motility and apoptosis. Smoking induces an imbalance in reactive oxygen species levels in seminal plasma by releasing cadmium (Cd), a metal toxicant (Ranganathan et al.)

5.1.5 Apoptosis

Apoptosis is a physiological activity in the testes that aims to destroy defective gametes and limit the population of gametes to match the capacity of Sertolli cells that support their maturation. A number of gametes have been found to produce FAS, which induces apoptosis by binding to FASL expressed by Sertolli cells. When FAS ligand (FASL) binds to its physiological receptor FAS, it activates caspase-8, which leads to cell death. Germ cell death is controlled by the FAS–FASL system (Passadaki et al.). However, a small fraction of faulty germ cells undergo sperm remodeling during spermiogenesis and present later in the

ejaculate with normal morphology but genetic changes (Burrello et al.). Apoptosis involves the rupture of cell membranes, cytoskeletal reorganization, nuclear condensation, and intranucleosomal DNA fragmentation (Kaufmann et al.).

5.2. Extrinsic Causes

5.2.1 Age

A mild decline in testicular volume with age represents a decrease in Sertoli cell mass, with global Sertoli cell function mostly, but not entirely, conserved at the expense of greater pituitary FSH stimulation (.Mahmoud et al.,2003).Increased sperm concentration, DNA fragmentation, and poor chromatin packaging, as well as a loss in semen volume, sperm morphology, and motility, are all linked to increased age in OAT patients (Plastira et al., 2007).Many hypotheses claim that ageing is caused by the buildup of unrepaired DNA lesions, which have been related to ageing in a variety of organs, including the brain, liver, and testis. Paul et al. discovered that oxidative stress in the form of 8-oxodG lesions causes an age-related buildup of DNA damage in the testis. Additionally, ageing appears to reduce germ cell capacity to repair DNA damage, resulting in the creation of spermatozoa with higher DNA damage.

5.2.2 Temperature of testis

The DNA integrity of spermatozoa can be harmed by scrotal heat stress. After scrotal heating and testosterone treatment the semen parameters, testicular histology, and germ cell apoptosis was mostly seen in round spermatids and pachytenespermatocytes (Wang et al,2007).

5.2.3 Abstinence

Abstinence length had a statistically significant favourable impact on sperm concentration and volume, as well as the quantity of leukocytes, but a statistically significant detrimental impact on sperm motility and vitality. With longer periods of abstinence, the percentages of DNA fragmentation and MMP (mitochondrial damage) increased. Abstinence also increase the proportion of sperm protamination(Comar et al, 2017).

5.2.4 Infections

In compared to fertile controls, patients with genitourinary infection by Chlamydia trachomatis and Mycoplasma have more fragmented sperm DNA. This rise is proportionally bigger than the effect on traditional semen characteristics, and it could lead to a reduction in reproductive potential. Antibiotic therapy appears to be useful in treating infection-induced elevated levels of DNA fragmentation (Gallegos et al., 2008).

5.2.5 Varicocele

Varicocele causes the formation of spermatozoa with less compacted chromatin, which is one of the potential reasons of infertility (Talibi et al., 2008)

5.2.6 Environmental and occupational toxicants

There is strong evidence that occupational exposure to various chemicals such as solvents, soldering fumes, epoxy and phenolic resins, heavy metals, or gasoline causes transgenerational cancers of various organs in the progeny in men in a variety of industries (chemical, painting, printing, electronics, or motor vehicle industries) (Lowengart et al., 1987; Wilkins and Koutras, 1988; Johnson and Spitz, 1989; Wilkins and Hundley, 1990). Even traffic contaminants like lead, sulphur oxides, and carbon monoxide have been proven to have a negative impact on sperm motility and functional testing (De Rosa et al., 2003).

5.2.7 Cryopreservation

Cryopreservation, commonly known as an applied component of cryobiology or the study of life at low temperatures, is the method that allows cells to be stabilised at cryogenic temperatures. Many developments in cryopreservation technology have led to the development of ways for maintaining a range of cell types at low temperatures, including male and female gametes. Cryopreservation has the ability to modify the structure and function of sperm. Several harmful mechanisms, such as heat stress with the development of intracellular and extracellular ice crystals, cellular dehydration, and osmotic shock, have been widely described during the freezing and thawing of human spermatozoa (Di Santo et al.).After cryostorage, sperm motility and viability both decreased significantly. Furthermore, after cryopreservation/thawing, sperm DNA fragmentation and DNA oxidative damage increased t significantly(Zribi et al.)

5.2.8 Sperm preparation techniques for ART

Centrifugal pelleting of unselected spermatozoa for ART has been known to cause sperm damage(Younglai et al,2001).The fact that centrifugal pelleting of spermatozoa may result in the formation of reactive oxygen species (ROS) 2–5-fold above baseline within 5 minutes, which is responsible for DNA damage, is one possible mechanism for this effect. (Aitken and Clarkson,1988).

5.2.9 Lifestyle

Tobacco smoking and drug abuse have negative health consequences, including a negative impact on male fertility (Bracken et al., 1990; Vine, 1996), but other lifestyle factors such as alcohol, caffeine consumption (Curtis et al., 1997; Anderson et al., 2010), psychological stress (Fenster et al., 1997; Zorn et al., 2008), nutrition, and physical activity are still suggestive and more research

is needed (Klonoff-Cohen, 2005). Obesity (Hammoud et al., 2008; Paasch et al., 2010) and age (Singh et al., 2003; Vagnini et al., 2007) have, on the other hand, been shown to have a negative impact on male fertility.

5.2.10 Chemotherapy and Radiotherapy

Radiation at high levels can cause permanent azoospermia by killing all of the spermatogonial stem cells. Only approximately 15% of patients recover sperm count or fertility following single doses of about 10 Gy, for example, although these results may be influenced by the fact that these patients also received cyclophosphamide(a strong chemo drug), which has some gonadal toxicity(Meistrich, 2013).

6. Methods of Detection

SCSA, Comet, SCD, and TUNEL are the four primary SDF tests described above that provide credible information on sperm DNA integrity in subfertility (Esteves et al., 2021).

6.1 SCSA

The DNA fragmentation may usually be observed before metaphase chromosomal changes in embryos. As a result, DNA damage measured by the Sperm Chromatin Structure Assay (SCSA) is regarded as the molecular precursor to subsequent large chromosome damage found under the light microscope((EVENSON and WIXON, 2005). The Sperm Chromatin Structure Assay (SCSA) is a test that measures clinically significant sperm nuclear chromatin integrity features. The assay makes use of the metachromatic properties of Acridine Orange (AO), a DNA probe, as well as flow cytometry principles (FCM) (Evenson and Jost, 2000).

6.2 COMET Assay

The sperm nucleus lacks protection against oxidative stress and is susceptible to oxidation-mediated DNA damage, making DNA damage a helpful tool in the diagnosis of male infertility. The Comet assay, also known as single-cell gel electrophoresis, is a quick and accurate method for detecting strand breakage in individual sperm DNA. Sperm cells are embedded in a thin layer of agarose on a microscope slide and lysed with detergent in high salt conditions during this method. The nucleus forms a nucleoid-like structure with supercoiled loops of DNA after this process eliminates protamines and histones. When examined under a fluorescent microscope, alkaline pH conditions cause double-stranded DNA to unwind, and subsequent electrophoresis causes the broken strands to migrate towards the anode, generating a comet tail. The luminous intensity of the head and tail reflects the amount of DNA present. The amount of DNA damage is measured by the relative fluorescence in the tail compared to the head (Simon et al., 2012).

6.3 SCD Assay

The SCD test is based on the principle that sperm with fragmented DNA do not create the typical halo of dispersed DNA loops seen in sperm with non-fragmented DNA after acid denaturation and removal of nuclear proteins, as shown in sperm with non-fragmented DNA. The study of DNA fragmentation using the particular DNA Breakage Detection-Fluorescence In Situ Hybridization (DBD-FISH) technique, which detects DNA breaks in lysed sperm nuclei, verified this. Sperm suspensions were implanted in an agarose microgel on slides and treated with 0.08 N HCl and lysing solutions containing 0.8 M dithiothreitol (DTT), 1% sodium dodecyl sulphate (SDS), and 2 M NaCl. Then, using fluorescence and brightfield microscopy, the percentages of sperm with nondispersed and dispersed chromatin loops were determined. According to the findings, all sperm with nondispersed chromatin had DNA fragmentation, as determined by DBD-FISH.As a result, the SCD test is a powerful and adaptable tool that may be used in both basic and practical research on human sperm DNA damage and organisation, in addition to facilitating straightforward sperm DNA quality assessment in the clinic.The commercialization of the SCD test (Halosperm®) (FERNANDEZ et al., 2005) offers professionals with a user-friendly tool for routine usage; the assay is now used in a variety of clinical applications (Fernández, Johnston and Gosálvez, 2018).

6.4 TUNEL Assay

The TUNEL assay is a useful tool for assessing DNA damage. Semen samples are routinely examined under a microscope to determine their capacity to fertilise. The TUNEL assay, in addition to routine semen analysis, can offer information on the extent of DNA damage present in a sample. TUNEL uses Terminal DeoxynucleotidylTransferase (TdT), a template-independent DNA polymerase that transfers deoxyribonucleotides to 3 hydroxyl (OH) single- and double-stranded DNA non-preferentially. The TdT enzyme adds deoxyuridine triphosphate (dUTP) to the free 3 -OH break-ends of DNA as a substrate. The additional dUTP can either be directly labelled, acting as a direct marker of DNA breaks, or the signal can be amplified by using a modified dUTP to which a labelled anti-dUTP antibody can be adsorbed. More label is integrated into a cell when there are more DNA strand break sites. Microscopically, the TUNEL-stained cells can be seen(Sharma, Iovine, Agarwal and Henkel, 2020).

7. Discussion

Sperm DNA fragmentation assays provide therapeutically meaningful information for natural conception and artificial reproduction that is distinct from that generated from standard semen parameters(concentration, motility, and morphology)but these criteria provide an overall picture of sperm quality, but they leave out one of the most critical aspects of the reproductive processi.e. DNA. Single or doublestrand DNA breaks can determine whether a male is fertile or infertile. Many intrinsic and extrinsic causes are responsible for sperm DNA fragmentation.The growing understanding of the impact of paternal variables on pregnancy outcomes, as well as advancements in treatment options, indicates the testing of sperm DNA fragmentation in infertile couples (Agarwal et. al). SDF can now be evaluated using a variety of techniques. TUNEL and SCSA are two of the most often used

procedures for detecting SDF. Despite the fact that the two procedures provide similar results, they disclose different sorts of damage. In vitro fertilisation and intracytoplasmic sperm injection, DNA fragmentation evaluated by SCSA is not associated to fertilisation rates, embryo quality, or pregnancy rates, according to previous studies, although it may be connected to spontaneous abortion rates. Greco et al. found that microinjection of sperm with DNA fragmentation greater than 15% as measured by TUNEL resulted in a pregnancy rate of 5.6 percent versus 44.4 percent when DNA fragmentation was less than 6 percent. The COMET (a single-cell gel electrophoresis test) and the SCDt (a simple approach for detecting DNA damage in individual cells) are two more tools often utilised in clinical research. The Halosperm® kit is a simple, cost-effective, quick, reliable, and accurate approach for detecting human sperm DNA fragmentation in the clinical andrology laboratory in routine process.

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