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REVIEW ARTICLE

SPERM DNA FRAGMENTATION: A NARRATIVE REVIEW

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Abstract

Objectives: This study examines the impact of sperm DNA fragmentation on male fertility, pregnancy outcomes, the success rates of assisted reproductive technologies (ART), and the available treatments. **Methods:** This study is a narrative review focusing on infertility, "sperm DNA fragmentation" and "sperm count" using papers published in PUBMED, Scopus, and Google Scholar from 2019 to 2022 in Spanish, Portuguese, and English. **Results:** A total of 29 studies were selected, demonstrating that reactive oxygen species play a central role in sperm DNA damage, impairing fertilization. Sperm DNA fragmentation is further influenced by factors such as oxidative stress, leukospermia, advanced paternal age, and environmental pollutants. **Conclusions:** While ART can help overcome some of the challenges posed by DNA fragmentation, it remains a significant barrier to fertility.

Keywords: Infertility; Sperm Count; sperm DNA fragmentation

Introduction

Infertility affects more than 180 million people worldwide with the male factor responsible for approximately 10% in all couples infertility and 50% of infertility overall.[1] Male infertility is often overlooked, contributing to unexplained infertility in 50% of cases, particularly in assisted reproduction due to increased sperm DNA fragmentation, a subject of ongoing debate.[2] The influence of DNA on reproduction was first demonstrated in bulls and cows in the 1990s, when reproductive outcomes were noted after induced damage to the DNA of gametes.[3] The DNA fragmentation index indicates a statistical threshold beyond which there is a longer time to achieve spontaneous pregnancy, reduced effectiveness of artificial insemination, a higher necessity for in vitro fertilization (IVF) cycles, and an increased risk of miscarriage.[4] Sperm DNA fragmentation increases with age, starting at reproductive age and doubling between 20 and 60 years.[5] This is due to the longer exposure time of individuals to endogenous and exogenous factors, further increasing the reactive oxygen species and causing damage to the genetic material[6]. Sperm's main role is to transport intact paternal DNA to the egg using a compact nucleus with protamines[7]. In the final spermatogenesis stage, the sperm reduces cytoplasm, becoming mobile but vulnerable to DNA damage from free radicals, despite protective chromatin organization.[2] This study will examine the impact of sperm DNA fragmentation on male fertility, pregnancy outcomes, the success rates of assisted reproductive technologies (ART), and the available treatments.

Methods

This study is a narrative review that explores the keywords "infertility," "sperm DNA fragmentation," and "sperm count." The review was based on a selection of peer-reviewed articles published between 2019 and 2022 in Spanish, Portuguese, and English. Relevant studies were identified through systematic searches in the following databases:

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PUBMED, Scopus, and Google Scholar. The inclusion criteria focused on studies addressing the impact of sperm DNA fragmentation on male fertility.

Results/Discussion

Free radicals

Free radicals, byproducts of oxygen metabolism, have dual roles in cellular processes like sperm maturation and fertilization. When their levels surpass the body's defense mechanisms, DNA damage occurs. Sperm's small cytoplasm makes them vulnerable to lipid peroxidation due to the high polyunsaturated fat content in the membrane. This compromises membrane function, ATP release, and flagellar movement, reducing sperm viability, motility, and fertilization potential.[8]

Oxidative damage

Oxidative damage can cause gene mutations, affecting embryo development or leading to later abortion if not repaired by oocyte enzymes before the zygote's S phase. Reproductive success hinges on the balance between sperm DNA damage and oocyte repair. Increased DNA fragmentation results from imbalances in protamine efficiency, free radical release, and DNA repair failures.[9]

Leukospermia

Leukospermia, as per WHO, is when white cells in ejaculate exceed $1x10^6$ concentration. It often stems from infections in the prostate and seminal vesicles, triggering a significant rise in reactive oxygen species (ROS) due to defense mechanisms involving NADPH. This inflammation lowers antioxidants and increases anti-inflammatory mediators.[8] However, it remains a very contradictory topic if it really is responsible for male subfertility.

In a meta-analysis published by Castellini et al.[10] in 2019, researchers showed little potential in changing fertilization rates after using assisted reproduction techniques (ART). It showed that there were no differences in oocyte fertilization with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), regardless of having leukospermia or not.[9] Leukocyte presence in semen doesn't affect fertility in assisted reproduction, miscarriages, or ectopic pregnancies. However, high peroxidase levels ($>4x10^6$) can harm IVF outcomes, since it often leads to reduced sperm motility and DNA.[10]

Advanced paternal age

Although there is no consensus on the definition of advanced paternal age, the most commonly used criterion is men over 40 years of age. Studies have shown that as men age, the mechanisms of DNA remodeling diminish, leading to defective spermatozoa. Most articles surveyed indicate an increase in DNA fragmentation with advanced paternal age.[11] However, when IVF is utilized, outcomes tend to improve even with increased DNA fragmentation.

Body Mass Index (BMI)

Obesity affects two-thirds of both developed and developing countries. It is a significant factor that increases oxidative stress and consequently causes DNA damage in various cells, including gametes. However, studies evaluating the relationship between obesity and the sperm DNA fragmentation index have produced ambiguous results. A recent meta-analysis by Sepidarkish et al.[12] in 2020, which included 8,255 men, identified a potential association between obesity and sperm DNA fragmentation, but it was not statistically significant. The study's author advises caution in this conclusion due to the heterogeneity of the subjects, which may impact the final results[12].

Air pollution

Studies have suggested that semen quality has been declining for decades due to factors such as air pollution, electromagnetic waves, obesity, alcohol consumption, psychological stress, smoking, and others.[13] A comprehensive study conducted by Zhang et al.[13] in 2020, which included 4,562 men, demonstrated that higher concentrations of pollutants in the plasma were associated with lower seminal volume, reduced sperm concentration, and poorer morphology. Additionally, the study found an increased sperm DNA fragmentation index in men with higher concentrations of pollutants in their blood. These results revealed a strong correlation between elevated air pollution levels and increased DNA fragmentation index (DFI).[13]

Varicocele

Varicocele is an abnormal vein enlargement in the pampiniform plexus, which affects 15% of adult males and up to 80% of patients with secondary infertility. It is linked to increased reactive oxygen species (ROS) production, damaging sperm DNA and increasing DNA fragmentation rates in the semen.[14] Research on varicocelectomy's role in reducing oxidative stress has grown in the past decade, as it has been suggested as a potential way to improve fertility by decreasing DNA damage. In fact, a study published by Neto et al in 2021 showed an improvement in sperm DNA fragmentation rates after varicocelectomy in infertile men.[14]

A meta-analysis performed by Quiu et al in 2020 also corroborates the data from this latter one, clearly indicating that varicocelectomy is an effective intervention for those patients with low sperm DNA integrity, significantly improving DFI and promoting greater possibilities of spontaneous pregnancy.[15] In another systematic review study by Soetandar et al.[16] in 2022, based only on microsurgical varicocelectomy performed in the last 5 years, it was shown that there was improvement in the seminal parameter and in the sperm DNA fragmentation index after the intervention.[16]

In a meta- analysis conducted by Zhang et al.[17] in 2022, 12 studies were included, comprising 845 patients diagnosed with varicocele and 2,377 healthy controls. The analysis revealed a significant difference in DNA fragmentation index (DFI) between varicocele patients and healthy controls, with a standardized mean difference of 1.4 (95% CI:0.83-1.98, $p<0.0001$), using the random effects model.

Subgroup analyses were performed to address substantial heterogeneity among the included studies, based on study region (Brazil vs. Other countries), detection methods of DFI (TUNEL, Comet and SCSA), sample size (<50 vs. >50), and age (<30 vs.>30 years).The stability of the pooled results was verified through sensitivity analysis.

In conclusion, patients diagnosed with clinical varicocele exhibited higher DFI than healthy controls, indicating that varicocele can impair sperm DNA and, consequently, the fertility potential of affected men.[17]

Assisted reproduction technique and DNA fragmentation index

Several studies have correlated DFI with pregnancy and fertilization rates following in Assisted Reproductive Technologies treatments. In a systematic review and meta-analysis performed by Simon et al.[2] in 2019, DIF was considered as a good outcome predictor in intrauterine insemination (IUI) and IVF; however, it was slightly less associated with ICSI outcomes. The DIF index was also negatively associated with embryonic development and positively correlated with miscarriage rates. Therefore, DIF might be considered closely related to male infertility regardless of other seminal parameters.[2]

Considering IUI, a study analyzing DIF and IUI outcomes concluded that couples with low DIF are three times more likely to conceive than those with higher DFI. However, this analysis must be done with care since the reproduced study used the same patient groups for the meta-analysis. Making it very clear the need for more confirmatory studies about.[18]

As for the use of IVF or ICSI related to DFI, 12,380 fertilization cycles were analyzed, considering implantation, pregnancy and live birth rates. The results indicated that higher DFI is related to negative results in IVF regarding implantation, pregnancy rates with a tendency to reduce live births, which corroborates many data from the literature.[19] However, regarding the ICSI results, no change was identified in the pregnancy and live birth rates. Despite this, more studies are needed to confirmation of the influence of DFI in ART outcomes, they still recommend the use of the DNA fragmentation test when opting for ICSI or IVF.[19]

Considering the good results obtained in ICSI, another article evaluated testicular sperm instead of ejaculate in men with high DFI. The results showed levels of evidence that the DFI has a correlation with outcomes in non-azoospermic patients.[20] Also, a relationship between recurrent pregnancy loss and DFI was observed, with statistical differences compared to the control group. Furthermore, it has been demonstrated in trials that there is a higher recurrence of pregnancy loss in couples with male factor with high DFI.[9] Collaborating with this data, a meta-analysis of 27 published articles also demonstrates recurrent abortions in groups with high DIF in trials.[20] The indication of carrying out DNA fragmentation tests in couples with repeated miscarriages is becoming increasingly clear.

Treatments

The treatments used to improve sperm vary greatly depending on the pathophysiology that affects the genetic material. However, changes in lifestyle, such as quitting smoking, weight loss, diabetes control, improving quality of life, and physical exercise, are essential for maintaining the molecular structure of the cell.

Physical exercise improves DNA damage due to its antioxidant-releasing property. Therefore, it results in the improvement of seminal parameters and male fertility.[21] Studies have also shown that high-intensity exercise can significantly improve DNA integrity. A randomized controlled trial evaluating patients who underwent 24 weeks of high-intensity training with a controlled diet, it was seen an improvement in oxidative stress and inflammation, decreasing DIF and improving other seminal parameters. It was concluded that high-intensive exercise improves reproductive function by decreasing oxidative states and anti-inflammatory status.[22] Other studies show a decrease in CD 14+, CD 16+, TNF alpha and liposaccharides after 12 weeks with high intensity training, leading to an improvement in sperm concentration, morphology, motility and decrease in DFI.[22]

In a pilot study conducted in Denmark by Humaidan et al.[23] in 2022, 93 patients with unexplained infertility were treated with oral antioxidants based on multivitamins (coenzyme Q10, omega-3 and trace elements) for a 3 months period. After these 3 months, which had also intervention programs to a healthier lifestyle, the DFI was significatively improved, but no changes were seen in the other seminal parameter or in the static oxidation-reduction potential.[23]

Antioxidant use is still debatable, but some studies show benefits for infertility. One study found nearly double spontaneous pregnancy rates, with no significant changes in live births or miscarriages.[24] It has also been shown a semen parameter improvement (concentration, motility, morphology), but not DFI changes with the use of antioxidants.[24] Combining antioxidants with varicocelectomy also seems to improve semen patterns.[24] Overall, antioxidants had a positive, low to moderate impact on pregnancy rates and seminal parameters but a low impact on total oxidizing capacity.[24]

There are also experimental studies with new drugs to improve sperm pattern, such as the use of astaxanthin 16 mg orally; however, a randomized double-blind study conducted by Kumalic et al.[25] in 2020 for 3 months in oligospermic, asthenozoospermic and teratozoospermic patients showed no improvement in DFI or spermogram parameters compared to placebo.[25]

Letrozole has also been investigated in patients who have higher DFI with the use of 2.5mg orally for 3 months. In this study, there was a statistical increase in concentration, motility and morphology after treatment. It was also observed to increase testosterone (T) and decrease estradiol (E2) levels with a T: E2 ratio of 1600%, when letrozole was administered in patients with T: E2 lower than 10. However, no improvement in ROS and body mass index levels were observed after treatment. On the other hand, a reduction in protamine deficiencies in sperm DNA with an improvement of up to 20% in spontaneous pregnancies has been observed.[26]

As for the combined use of human chorionic gonadotropin and menopausal chorionic gonadotropin, a randomized double-blind study was performed for 3 months in men with idiopathic oligozoospermia. A noted improvement in seminal parameters was observed, as well as a decrease in DFI in groups that had moderate and high DFI. Additionally, there was increase in spermatid concentrations and an enhancement in spontaneous pregnancy rate in groups with poorer baseline fertility.[27]

Experimental studies involving traditional Chinese medicine using ram herbs for health and safety are being conducted; however, there is still no conclusive evidence supporting their use.[28]

Another additional point that has been developed for the improvement of DFI are drug therapies in patients with changes in seminal parameters, such as antioxidant blend tablets. Patki et al.[29] showed a prospective, double- blind, randomized, placebo- controlled trial with 300 sub- fertile males aged 25-45 years across ten study sites in India. Participants were randomly assigned to receive either an antioxidant blend or a placebo, The study assessed changes in sperm count, motility normal morphology, semen volume, and DNA fragmentation index over a 90-day treatment period.

A post hoc analysis further stratified the data based on different criteria. The treatment group showed significant improvements in sperm count, semen volume, sperm motility, and normal sperm morphology. Severe oligospermia subjects (sperm count < 5 million/mL, 5- 10 million/mL and 10.1-15 million/mL) and those with high to extremely high baseline DFI (20-30%, 31-40%, and above 40%) also showed improvements in sperm count.

The results confirmed that antioxidants are effective in reducing oxidative stress, improving sperm DNA integrity, and enhancing semen parameters, particularly in males aged 40 and above.[29]

Conclusion

Free radicals and oxidative damage significantly impact male fertility by increasing sperm DNA fragmentation, often exacerbated by factors like leukospermia, varicocele, obesity, and environmental pollutants. Lifestyle modifications, varicocelectomy, antioxidants, and high-intensity exercise can improve sperm parameters and DNA integrity. ART methods, particularly ICSI, help mitigate adverse effects, though outcomes vary. Ongoing research is essential to refine treatments and enhance fertility success rates.

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