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ORIGINAL PAPER

Sperm DNA fragmentation: Focusing treatment on seminal transport fluid beyond sperm production

Moises Abraham Adel Domínguez [1], Walter D. Cardona Maya [2], Andrés Mora Topete [1]

[1] Centro de Uro-Andrología S.C., Guadalajara, Jalisco, México;
[2] Grupo Reproducción, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.

Summary ***Aim: To determine the effects of oral antibiotics and anti-inflammatory agents on semen parameters and the sperm DNA fragmentation index (DFI).***
Methods: Ninety-eight men with infertility diagnosis were included. The participants submitted two semen samples, before and at least two months after treatment. Macroscopic and microscopic semen parameters were evaluated following the World Health Organization (WHO) guidelines. In addition, the sperm DFI was evaluated using the sperm chromatin dispersion (SCD) technique. Subsequently, a treatment regimen was administered, including daily oral doses of ciprofloxacin (1000 mg) and doxycycline (100 mg) for 21 and 10 days, respectively. In addition, non-steroidal anti-inflammatory drugs (15 mg of meloxicam) were used for 10 days. After treatment, the same parameters were re-evaluated for new semen samples taken under the same initial conditions.
Results: After treatment, significant increases in pH, sperm count, total concentration, and normal sperm morphology were observed, but no significant differences were found in the seminal volume parameter or progressive motility. After treatment, a significant decrease in the concentration of immature cells was observed, and although not statistically significant, a reduction in the concentration of leucocytes was observed. After treatment, the mean sperm DFI significantly decreased from 28.24 ± 12.39% to 16.2 ±7.1%.
Conclusions: Treatment with antibiotics and anti-inflammatories significantly reduced the sperm DFI and improved semen quality.

KEY WORDS: *Male fertility; Infertility; DNA fragmentation index; Semen; Spermatozoa.*

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INTRODUCTION

Infertility is a problem that affects an increasing number of people around the world, regardless of gender (1, 2). When studying semen to evaluate and diagnose male fertility, it is considered that male factors are responsible for 50% of infertility cases (3), and specialists focus primarily on the macroscopic and microscopic parameters of semen (4). However, although these parameters can be considered essential for determining the success of a pregnancy, it is crucial that, in the presence of infertility, sperm functional alterations that could affect embryonic development are also evaluated (5-7). A high sperm *DNA fragmentation index* (DFI) has been associated with decreased male fertility and an increased risk of spontaneous abortions (8, 9). Therefore, it has become an essential parameter in fertility studies for evaluating sperm quality.

Sperm DNA fragmentation can occur for different reasons, such as oxidative stress, DNA lesions, infections, and other factors (10). Different techniques are used to evaluate the sperm DFI, including the single-cell gel electrophoresis (comet) assay, the sperm chromatin structure assay, *DNA breakage detection-fluorescence in situ hybridization* (DBD-FISH), and terminal deoxynucleotidyl transferase *deoxyuridine triphosphate* (dUTP) nick end labeling (TUNEL) assay, among others. These methodologies have shown promising results but require specialized equipment and high-cost reagents, making those tests unavailable for all populations (11). In contrast, the *sperm chromatin dispersion* assay (SCD) offers many advantages, such as its low cost compared to other techniques and its simplicity, practicality, speed, and reproducibility (11-13).

The most explored treatment for reducing sperm DFI is the chronic use of oral antioxidants, based on the evidence that DNA fragmentation is frequently associated with states of oxidative stress (14-16). However, the presence of bacteria in semen can also affect semen quality, and consequently, identifying the presence of bacteria in couples with fertility problems could be beneficial for improving semen parameters and increasing the chances of successful conception (17).

Therefore, antibiotics could also improve sperm DFI in some instances in which DNA fragmentation is caused by a bacterial infection in the male reproductive tract; treatment with appropriate antibiotics could clear the infection and reduce inflammation, which could help improve sperm DNA integrity (18).

This study aimed to determine the effects of oral antibiotics and anti-inflammatory agents on semen parameters and the sperm DFI.

MATERIALS AND METHODS

Ninety-eight men with the diagnosis of primary or secondary infertility, either directed for advanced and non-

advanced reproductive techniques were included (*Centro de Uro-Andrología S.C., Guadalajara, Jalisco, México*). Each patient was invited to participate in the study, and authorization was requested under current ethical standards; each participant also provided informed consent.
The study participants provided two semen samples under similar conditions of sexual abstinence (3-5 days), one before treatment and one at least two months after treatment. Both semen samples were analyzed in the same laboratory by the same expert technician.
Basic macroscopic and microscopic sperm parameters were evaluated following the WHO guidelines (19), and the sperm DFI was determined using the sperm chromatin dispersion test (SCD, Sperm DNA kit, Obi biotek) following the manufacturer's instructions. In addition, the concentrations of leukocytes and immature cells were also evaluated.
After the initial semen evaluation, the patients were invited to receive daily oral doses of ciprofloxacin (1000 mg) or doxycycline (100 mg) for 21 or 10 days, respectively. In addition, non-steroidal anti-inflammatory drugs (15 mg of meloxicam) were used for 10 days.

Statistical analysis

The data distribution was evaluated with the normality test of residuals, and the descriptive analysis was carried out according to the distribution of the variables. Paired t tests were carried out for related samples when the data met normality, and the Wilcoxon test was used for non-normal distributed samples. A p value of < 0.05 was considered to indicate statistical significance.

Results

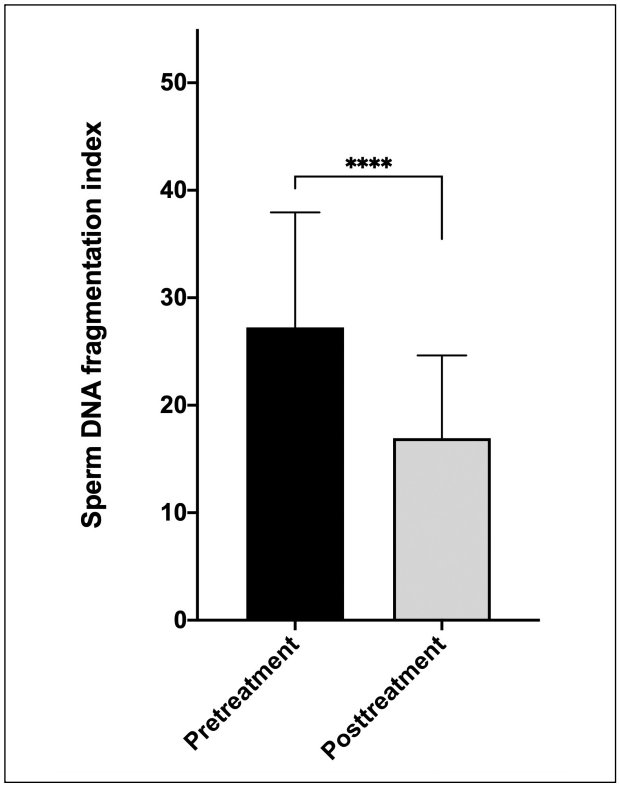
Ninety-eight men with infertility diagnosis participated in the study. The median age was 36 ± 6.7 years, with an interquartile range (25-75) of 32-41 years.
As shown in Table 1, no significant differences were found regarding seminal volume or progressive motility. However, after treatment, a significant increase in pH, sperm count, total concentration, and normal sperm morphology was observed.
In addition, a decrease, although not statistically significant, in the concentration of immature cells and leucocytes was observed after treatment: 1.4 ± 2.0 million/mL *vs.* 0.85 ± 1.6 million/mL (p = 0.2613) and 1.9 ± 2.2 *vs.* 1.2 ± 1.8 million/mL (p = 0.4541), respectively.

Table 1.
Changes in semen parameters before and after treatment.

	Pre-treatment	Post-treatment	P value
Volume, mL [1]	2.9 ± 1.6	3.0 ± 1.5	0,529
pH [2]	7.6 ± 0.6	7.7 ± 0.4	0.007
Progressive motility, percentage [2]	33 (10-50)	34 (21-50)	0.763
Concentration, 10^6/mL [2]	21 (8.7-50.8)	22 (9.2-58)	0.046
Total concentration, 10^6/ejaculate [2]	60 (20-126)	83.3 (19.1-172.5)	0.006
Normal morphology, percentage [2]	1 (1-2)	2 (1-4)	< 0.01

[1] *Paired t test, data show means ± standard deviations.*
[2] *Wilcoxon test, data show medians and their interquartile range (p25-75).*

Figure 1.
Changes in the sperm DFI after treatment.



Finally, after treatment, the mean sperm DFI significantly decreased from 27.2 ± 10.7% to 16.9 ± 7.6% ($p < 0.001$) (Figure 1).

Discussion

The results of this study support the effectiveness of an antibiotic and anti-inflammatory treatment approach in male patients diagnosed with infertility, consistent with previously published preliminary results (20). Initial evaluation of semen samples revealed various parameters essential for determining sperm quality, such as volume, pH, viscosity, sperm concentration and sperm DFI (7). We hypothesize that the sperm DFI problem lies not in the quality of the sperm itself during spermatogenesis but in the factors that promote the degradation of its integrity once it has reached maturity and in the seminal tract. Following this approach, we have implemented a treatment plan based on dual-scheme antibiotics accompanied by anti-inflammatories to improve the quality of the transport medium rather than the spermatogenesis process in the seminal fluid used for spermatogenesis.
A few years ago, there was no consensus on how to manage high *prostate-specific antigen* (PSA) levels that have occasionally been detected during PSA screening, because PSA levels can increase for several reasons, including trauma, ejaculation, rectal and urethral procedures, and numerous noncancerous etiologies such as benign prostatic hyperplasia, inflammation, and infection (21); therefore most urologists make decisions based on their training and experience, and the use of antibiotics to reduce high PSA is

an alternative (22), although it is not safe to discard biopsies in patients who achieve a satisfactory PSA response to antibiotics (22), because if it has been observed that the use of antibiotics delays the diagnosis of prostate cancer.
In the same way, most of the seminal fluid is composed of by secretions of accessory glands, which comprise the prostate, the seminal vesicles, and the bulbourethral glands, and it is possible that the presence of bacteria in this fluid and in its places of origin (23, 24) could negatively affecting the semen and sperm quality.
Therefore, as bacteria are present in the semen, safe from its origin, the use of antibiotics will eradicate the local inflammatory effect that promotes tissue damage and release of more prostate antigen, as is happening in patients with increased PSA. However, given that the fragmentation of sperm DNA don't occur during its formation, eliminating bacteria from the seminal pathways would allow the elimination of local inflammation and its deleterious effects on sperm DNA.
Bacteria may release soluble factors, such as lipopolysaccharides, hemolysins, and other soluble spermatotoxic factors that can affect sperm physiology (23, 25). The effects of bacterial infection may be multiple, comprising reduction in motility, induction of teratozoospermia (abnormal sperm morphology), apoptosis, DNA fragmentation, sperm agglutination, and exposure to oxidative stress through forming *reactive oxygen species* (ROS) (23, 25).
Altogether, the use of low-potency steroids is necessary because antibiotics do not affect the mucosa of the urothelium or the urethra, but steroids can generate an anti-inflammatory effect on mucous membranes (26). Therefore, using antibiotics and steroids can impact both the tissue of the epididymis, prostate and seminal vesicles as well as the urothelial mucosa of the urethra and prostatic urethra, where the semen is expelled.
A key finding in this study was the high sperm DFI observed in the samples, with a mean of 28.24 ± 12.39%. This result highlights the presence of significant sperm DFI in the infertile patient population. According to the literature, a cutoff of 20% can differentiate between fertile and infertile men (27). Previous evidence from a comparative study carried out with fertile and infertile patients showed that the average sperm DFI was 29.95% (26.6-34.3%) in infertile patients and 19.90% (15.6-24.4%) in fertile patients; moreover, a sperm DFI > 26.1% was associated with a 2.8-fold increased risk of infertility (28).
Furthermore, orally administered ciprofloxacin and etoricoxib for 15 days reduced the sperm DFI from 36 ± 3% to 24.9 ± 1% (20).
The implemented treatment, which included antibiotics and anti-inflammatories, led to significant changes in semen parameters. A significant increase in pH and total sperm concentration was observed at the end of treatment, suggesting an improvement in sperm quality. Although the semen volume did not significantly change, these other indicators support the effectiveness of the therapeutic approach (29).
Furthermore, a reduction in the concentration of leukocytes and round cells, which are markers of inflammation, is an important finding. Although the decrease in leukocyte concentration did not reach statistical significance, the reduction in the number of round cells was statistically significant. Since round cells are a marker of transient inflammation secondary to spermatogenic aggression, their decrease is positive and could be related to a decrease in sperm DNA fragmentation (30).
The most striking finding was the significant decrease in the DFI at the end of treatment.
This indicates a substantial improvement in sperm DNA integrity and, therefore, in sperm quality. Taken together, these results support the initial hypothesis that male infertility problems do not reside solely in the quality of the sperm themselves but rather in the factors that may promote the degradation of their integrity once they have reached maturity. Antibiotic and anti-inflammatory treatments effectively address these factors, substantially improving semen parameters, including significantly reduced sperm DFI (20).
The proposed hypothesis is that a significant percentage of sperm DFI can be generated during its passage through the seminal pathway rather than being an exclusive process of spermatogenesis. In essence, factors present in the epididymis, seminal vesicles, and prostate can substantially contribute to fragmentation, even in the absence of clinical symptoms or semen culture findings. These factors include inflammatory changes in the epididymis, asymptomatic bacterial colonization, subclinical prostatitis, infrequent ejaculation and others. Evaluating and managing these conditions in the seminal pathway could improve, to a certain extent, the quality of the seminal sample before deciding on *Intracytoplasmic sperm injection* (ICSI).
Therefore, in this way, the possibility of optimizing sperm quality through a therapeutic approach targeting the seminal pathway, as a strategy before considering assisted reproduction techniques, has to be considered, intending to improve natural pregnancy rates or reduce the need for ICSI in some cases.

Conclusions

These findings are promising and may have significant implications for managing male infertility aiming to the quality of the seminal transport fluid beyond spermatogenesis, in order to obtain a non-hostile environment where sperm DNA fragmentation is reduced. However, it is essential to highlight the need for additional research and long-term studies to validate these results and better understand the underlying mechanisms involved in this treatment.

Declarations

Ethical approval: *Authorization was requested under current ethical standards; each participant also provided informed consent.*

Availability of data and material: *All inquiries can be directed to the corresponding author.*

Competing interests: *None.*

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Correspondence

Moises Abraham Adel Domínguez, MD
moises.adel@gmail.com

Andrés Mora Topete, MD
drandresmora@hotmail.com
Centro de Uro-Andrología S.C., Guadalajara, Jalisco, México

Walter D. Cardona Maya, PhD
wdario.cardona@udea.edu.co
Grupo Reproducción, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia