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ABSTRACT

Traditional semen analysis remains the standard of clinical care to initially investigate and diagnose male infertility. However, the basic semen analysis does not provide information on the oxido-reductive potential (ORP) and nuclear integrity of semen and spermatozoa, respectively. Sperm DNA fragmentation (SDF) and oxidative stress are markers believed to be implicated in the pathogenesis of male infertility. Thus, here we were prompted to evaluate the relationship between ORP and SDF in infertile men receiving antioxidants. The cohort study included 64 men undergoing infertility evaluation. Sperm DNA fragmentation index (DFI) was measured with TUNEL assay and ORP in semen was assessed using the standardized MiOXSYS system. Patients were then divided into two study groups according to the use of antioxidant supplementation. There was no statistically significant difference in the mean abstinence period (3.0 ± 0.2 vs. 2.4 ± 0.1 days), sperm concentration ($64.9 \times 10^6/\text{mL}$ (8.1) vs. $55.4 \times 10^6/\text{mL}$ (10.4)) and DFI (15.8% (1.8) vs. 19.0% (2.3)) between groups ($p > 0.05$). However, mean ORP was significantly lower in the no antioxidant compared to the FertilPro antioxidant group ($1.0 \text{ mV}/10^6 \text{ sperm/mL} \pm 0.2$ vs. $1.6 \text{ mV}/10^6 \text{ sperm/mL} \pm 0.4$). We observed no significant correlations between SDF and semen ORP in the no antioxidant group ($r = -0.02$; $p = 0.89$) and the FertilPro groups ($r = -0.11$; $p = 0.58$). When applying the established clinical cut-off ($1.34 \text{ mV}/10^6 \text{ sperm/mL}$), only 6% of patients had both an abnormal SDF and ORP in the no antioxidant group and 17% of patients in the FertilPro group. These findings suggest that monitoring these markers in men with infertility may provide us with a better understanding of the complex relationship between semen.

INTRODUCTION

Infertility is characterized by an inability to conceive, despite repeated attempts for a period of more than one year. It is a condition that affects approximately 15% of couples of childbearing age worldwide. Although it may be of female origin, there are also several male factors that may be involved. In general, it is possible to diagnose male infertility via a semen analysis. In fact, low sperm count, altered morphology, or a reduction in the amount of effective movement can harm a couple's chances of conception. Although sperm parameters remain important for the diagnosis of fertility in men, the methods used are highly criticized due to their high level of variability. Indeed, despite the attempt of the World Health Organization (WHO) to standardize the practices, the results remain subjective and vary greatly depending on the observers as well as the handling errors, precision and variability within the specimens themselves. Furthermore, in several cases (30 to 50%), sperm parameters are not sufficient to establish the infertility diagnosis.

RESULTS

Table 1. The variation coefficients of intra-assay and inter-assay reproducibility of ORP using MiOXSYS

	Mean ORP (mV)	SD	CV (%)
Intra-assay	226.1 (6)	1.2	0.52
Inter-assay lot 1	242.5 (20)	1.8	0.76
Inter-assay lot 2	222.7 (20)	1.5	0.66
Inter-assay lot 1 and 2	232.6 (40)	10.2	4.39

*Number of data in brackets

Figure 1. Correlation between seminal ORP and sperm DNA fragmentation in patients following up at a fertility clinic with no history of antioxidant therapy

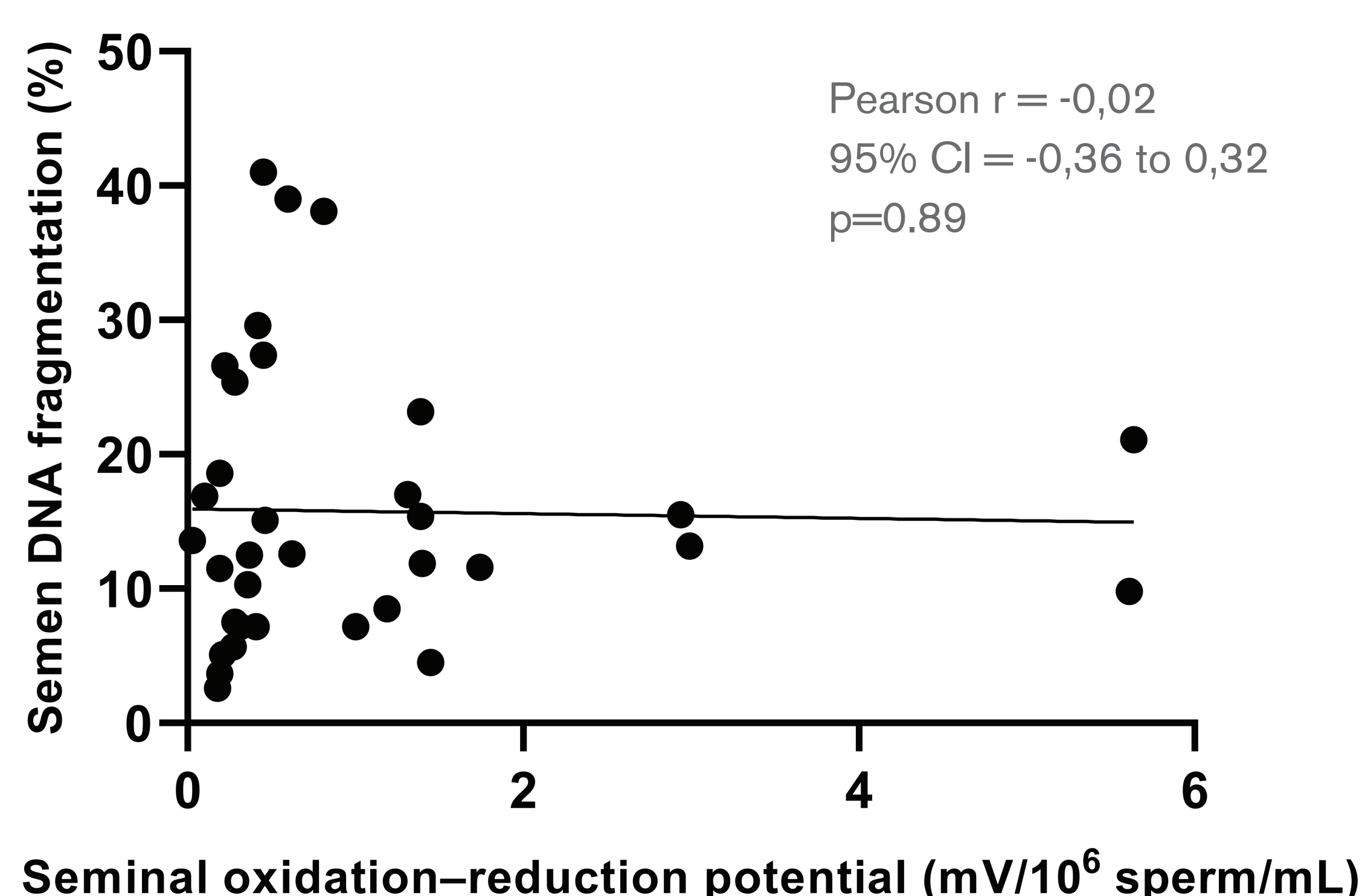
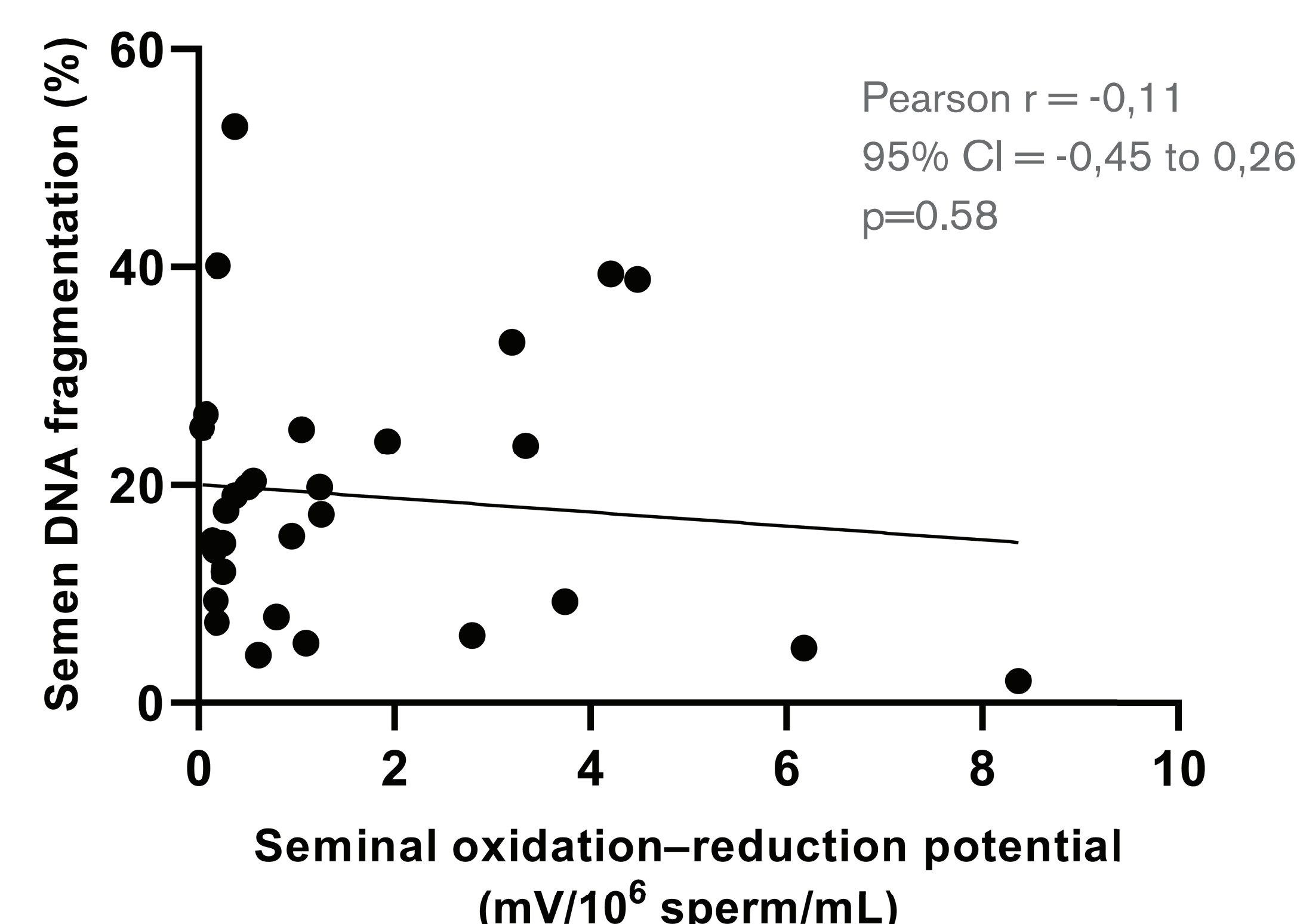


Table 2. Semen parameters in patients with no history of antioxidant therapy and in patients on FertilPro therapy

Patients	DFI (%)	ORP	Abstinence (days)	Concentration (mil/ml)
Control	15.8 (1.8)	1.04 (0.2)	3.0 (0.2)	64.9 (8.1)
FertilPro	19.0 (2.3)	1.63 (0.4)	2.4 (0.1)	55.4 (10.4)

*ORP is expressed in mV/10⁶sperm/mL

Figure 2. Correlation between seminal ORP and sperm DNA fragmentation in patients following up at a fertility clinic and taking FertilPro supplement



STUDY QUESTION

Does an elevated ORP ($\geq 1.34 \text{ mV}/10^6 \text{ sperm/mL}$) is associated with higher SDF amongst infertile patients and should it be measured routinely to assess the reproductive potential?

METHODS

Study design: The cohort study included 64 male patients undergoing infertility evaluation. Following the reception of written informed consent, the sperm DNA fragmentation index (DFI) was measured with terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL) assay (AccuriC6 Flow Cytometer) and ORP in semen was assessed using the standardized Male Infertility Oxidative System (MiOXSYS) system. Patients were then divided retrospectively into two study groups according to the use of antioxidant supplementation: no antioxidant or FertilPro antioxidant therapy group.

Participants: Out of the 64 males included in the study, 34 had no history of antioxidant therapy and 30 were taking FertilPro on a regular basis prior testing.

Settings: All sperm samples were analyzed according to the WHO criteria, and a cut-off value of 16.9% for DFI and $1.34 \text{ mV}/10^6 \text{ sperm/mL}$ for ORP were applied to classify normal vs abnormal samples.

Quality control: ZoBell's Solution, Oxidation-Reduction Potential Standard (988016, Ricca Chemical) was used as a positive control. A solution of ascorbic acid in SAGE 1-Step HSA medium (7701, Origio) served as a negative control. A calibration solution at pH 7 saturated with quinhydrone (ORPCALKIT, Myron L), was used as an extra control for the accuracy of the results.

Data analysis: Pearson's r was used for correlation analysis and continuous variables, or percentage results are expressed as mean \pm (standard error).

CONCLUSIONS

Semen DFI doesn't correlate with ORP in patients undergoing routine screening for infertility or in patients on FertilPro antioxidant therapy. This finding highlights the importance of testing both semen ORP and DFI for screening, clinical diagnosis, and antioxidant therapy monitoring especially in patients with unexplained infertility.

Limitations: The sample size of the current study was moderate, despite similar observation between both study groups. The effect of antioxidants on both semen ORP and DFI should be confirmed in a prospective controlled trial.

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