Multidisciplinary

The effect of sexual abstinence period on 8-hydroxydesoxyguanosine, nitric oxide and total antioxidant capacity in seminal plasma and semen quality in abnormal semen

Yousra Amar Eedan[1] and Sahib Yahya Al-Murshidi[1]

[1] Department of Laboratory Analysis, Faculty of Science, University of kufa, Iraq

Correspondence Author: Yousra Amar Eedan

Received 12 May 2024; Accepted 25 Jun 2024; Published 6 July 2024

Abstract

Background: This study aimed to evaluate the influence of (2-3), (4-5) days periods of sexual abstinence on semen parameters and on levels of both oxidative stress and antioxidants in seminal plasma of abnormal semen.

Methods: Abnormal semen samples were collected from 47 infertile men and their semen parameters and levels of NO, TAC, 8-OHdG were examined in both periods (2-3) days and (4-5) days.

Results: The results of the current study showed a statistically no significant ($p \leq 0.05$) decrease in sperm concentration and normal sperm morphology of a group of (4-5) days compared to the group (2-3) days, and no differences was observed in the curvature of progressive motility, semen volume and pH between the two groups and showed a statistically no significant ($p \leq 0.05$) increase in TAC concentration and also a statistically no significant ($p \leq 0.05$) decrease in the level of 8-OHdG in the seminal plasma of the abstinence group (4-5) days compared to the abstinence group (2-3) days.

Conclusion: A sexual abstinence periods (2-3) days is appropriate for a patient with abnormal sperm, because an increase in the period of abstinence (4-5) days leads to a decrease in sperm concentration and the percentage of normal sperm morphology, despite the improvement in the level of TAC and 8-OHdG.

Keywords: total antioxidant capacity, sexual abstinence period (2-3), (4-5) days, 8-hydroxydeoxyguanosine, semen quality, nitric oxide, semen

1. Introduction

The seminiferous tubules create human sperm, which is subsequently stored in the epididymis for later release [1, 2]. The male mammalian gamete must transit through the epididymis, where it experiences a number of physiological and biochemical changes, in order to mature and gain fertilization potential [2].

Three main reasons may be the cause of the variations observed in human semen samples over time, according to studies. pre-analytical factors, such as intrinsic biological variation, analytical randomization (precision), systematic error (bias), and the length of the sample's journey to the lab (in the case of semen) [3-6]. Spermatozoa accumulate in the epididymis after prolonged abstinence. As a result, spermatozoa are vulnerable to oxidative stress, which has been linked to reduced sperm motility, lipid peroxidation, DNA damage, and lowered rates of fertilization [7, 8].

There was an estimate of 2 to 11 days for the epididymal transit time [1]. A shorter period of sexual abstinence was associated with a higher percentage of normal sperm morphology in the teratozoospermic group [9] demonstrated an increase in sperm concentration in the second ejaculation after a short period of abstinence in patients. Those suffering from oligozoospermia, asthenozoospermia, and teratozoospermia [10-13]. Male infertility is diagnosed through semen analysis using the World Health Organisation's reference limits [6]. As per the existing recommendations, it is recommended that semen samples be obtained for examination following a minimum of two days and a maximum of seven days of refraining from sexual activity. As required by the American Society of Reproductive Medicine (ASRM) and the American Urologic Association (AUA), the usual methodology for semen analysis collection involves a two- to seven-day abstinence period prior to clean collection by masturbation [14,15,6].

This study aimed to evaluate the influence of (2-3), (4-5) days periods of sexual abstinence on semen parameters and on levels of both oxidative stress and antioxidants in seminal plasma of abnormal semen.

2. Materials and methods

2.1 Patients

The present study was conducted in specialized infertility laboratory in Al-Najaf governorate. This work was carried out between July 2023 and May 2024. Semen samples were taken from fertility centre patients who were abnormal semen (Oligoasthenozoospermic, Asthenozoospermic, Oligozoospermic, Asthenoteratozoospermic, Oligoasthenoteratozoospermic) were collected from 47 infertile men. results of semen analysis (all semen and sperm parameters) and biochemical tests which included total antioxidant capacity, nitric oxide, and 8-OHGD of both periods (2-3) days and (4-5) days. The semen samples were collected in clean and dry containers and they were put in an incubator at 37C for 30 to 1 hour to complete liquefaction time of seminal fluid. After complete of liquefaction time for semen samples, the semen samples were analyses according to WHO 2010 and

WHO, 2021 guidelines to determine semen parameters, and remaining samples were centrifuged to separate seminal plasma from semen samples.

Determination of TAC (Total Antioxidant Capacity) in seminal plasma

Using assay kit (FRAP Method) (spectrophotometer / Microplate reader, NO. BC1315, 96T, Tongzhou, Beijing, China). The colourless oxidised Fe+++ form is converted into a blue-colored Fe++ tripyridyltriazine molecule by the action of electron-donating antioxidants. This process is measured by the FRAP assay.

Determination of 8-OHDG (8-Hydroxydeoxydeoxyguanosine) in seminal plasma

Using an enzyme-linked immunosorbent assay (ELISA kit; NO.85A, 96T, Tongzhou, Beijing, China), the amounts of 8-OHdG in 50 µL of seminal plasma were determined. After that, an ELISA reader was used to detect the colour reaction product at 450 nm, and a standard curve was used to determine the quantity of 8-OHdG. The ultimate outcomes were presented in ng/mL.

Determination of NO (Nitric Oxide) in seminal plasma

Using assay kit chemical method (spectrophotometer / Microplate reader, NO. BC5485, 96T, Tongzhou, Beijing, China). NO is easily oxidized to NO_2 in vivo or in aqueous solution. Under acidic conditions, NO and diazonium salt sulfonic acid amine form diazo compounds, further coupled with naphthyl vinyl diamine. The product has a characteristic absorption peak at 550 nm, the absorbance value is measured, and the content can be calculated.

2.2 Statistical analysis

The data of this study were analyzed using the MedCalc software (version 14.25). The Independent Sample t-test, expressed as (Mean±SD), was employed to compute differences between them. P-values below 0.05 were regarded as statistically significant.

3. Results

The effect of a sexual abstinence period on abnormal semen group semen quality

The results of the current study showed a statistically no significant ($p \leq 0.05$) decrease in sperm concentration and normal sperm morphology of a group of (4-5) days compared to the group (2-3) days, and no differences was observed in the curvature of progressive motility, semen volume and pH between the two groups.

Table 1: Semen parameters according to sexual abstinence periods in abnormal semen group

Semen parameters	2-3 days (n=20)	4-5 days (n=27)	*p* value
Sperm concentration (Million/ml)	22.550±22.952	16.611±17.646	0.321ns
Progressive motility (%)	21.800±17.489	21.037±17.423	0.883ns
Normal sperm morphology (%)	41.250±12.388	33.111±18.048	0.090ns
Semen volume (ml)	3.455±1.191	3.333±1.407	0.756ns
Round cell (Million/ml)	1.950±2.605	2.519±2.260	0.428ns
PH	8.060±0.614	8.048±0.642	0.949ns

Data represented as Mean(±SD), ns = not significant at $p \leq 0.05$

The effect of sexual abstinence period on oxidative stress biomarkers (8-hydroxydesoxyguanosine, nitric oxide, total antioxidant capacity) in seminal plasma of abnormal semen group

The results of the current study showed a statistically no significant ($p \leq 0.05$) increase in TAC concentration and also a statistically no significant ($p \leq 0.05$) decrease in the level of 8-OHdG in the seminal plasma of the abstinence group (4-5) days compared to the abstinence group (2-3) days to abnormal semen samples.

Table 2: Oxidative stress biomarkers according to sexual abstinence periods in abnormal semen group

Oxidative stress markers	2-3 days (n=20)	4-5 days (n=27)	*p* value
Total Antioxidant Capacity (T-AOC) (µM/L)	0.065±0.013	0.071±0.012	0.114ns
Nitric Oxide (NO) (µmol/L)	0.035±0.023	0.032±0.016	0.621ns
8-OHdG ng/mL	101.393±72.174	72.509±64.015	0.154ns

Data represented as Mean(±SD), ns = not significant at $p \leq 0.05$

4. Discussion

According to the current study's findings, the length of the period of sexual abstinence for the abnormal semen group was associated with a decrease in sperm concentration and a percentage of normal sperm morphology. The comparison was between periods of sexual abstinence from 2 to 3 days and from 4 to 5 days. Here, the results we reached agreed with the study, which indicated that a shorter period of sexual abstinence was associated with a higher percentage of normal sperm morphology in the teratozoospermic group [9], whereas the group with abnormal sperm in our study included the teratozoospermic group.

The benefit of a shorter period of sexual abstinence was also noted in other studies investigating at abnormal semen [16]. But conflicting results exist, some studies show that there is no correlation between the time of sexual abstinence and the

change in the percentage of normal shape of sperm in normal and abnormal semen samples [17]. This may be due to the negative effect of ROS levels on the percentage of sperm with normal shape [18]. Increased exposure to epididymal ROS may lead to a decrease in the number of normal sperm morphology after a long period of sexual abstinence.

Our results also agreed with the study that indicated an improvement in the sperm concentration of the abnormal group during a period of sexual abstinence of less than two days [9]. They concurred with earlier research as well, showing that patients' sperm concentration increased in their second ejaculation following a short period of abstention. Those suffering from oligozoospermia, asthenozoospermia, and teratozoospermia [10-13].

At the same time, we observed an increase in the number of round cells with a prolonged period of sexual abstinence for the abnormal group. Our results agreed with the study that indicated that a low concentration of Leukocytes could help improve semen parameters such as sperm concentration, movement, and shape [19, 20], that there is an inverse relationship between rounded cells and semen parameters.

5. Conclusion

A sexual abstinence periods (2-3) days is appropriate for a patient with abnormal sperm, because an increase in the period of abstinence (4-5) days leads to a decrease in sperm concentration and the percentage of normal sperm morphology, despite the improvement in the level of TAC and 8-OHdG.

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