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Redox status assessment in infertile patients with non-obstructive azoospermia undergoing testicular sperm extraction: A prospective study

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Abstract

Background: Oxidative stress (OS) is one of the most prevalent causes of sperm damage, through the toxic effects of endogenously generated hydrogen peroxide, superoxide anion, and hydroxyl radicals. Peripheral leukocytes represent a feasible model for studying the pathophysiology of OS-mediated homeostasis, which can be responsible for cell dysfunction and cell injury.

Objective: To evaluate the redox status in patients with non-obstructive azoospermia (NOA), establishing the potential role exerted by reactive oxygen species (ROS) in the genesis of testicular secretory injury.

Material and methods: From May 2018 to March 2019, 39 patients were enrolled in this prospective single-center cohort study and divided into two groups. Group 1 included 19 patients with NOA, and Group 2 included 20 normozoospermic men, partners of women with infertility tubal factor. All patients underwent serum blood tests. NOA underwent testicular sperm extraction (TeSE). ROS production (in lymphocytes, monocytes, and granulocytes) was assessed by fluorescence-activated cell sorting (FACS) analysis. Plasma oxidative stress was evaluated by lipid peroxidation markers (MDA) and total antioxidant capacity (TAC) both assessed by fluorometric techniques.

Results: Mean lymphocyte ROS production resulted 967.0 ± 224.5 vs 728.0 ± 98.0 (NOA vs Controls, $P < .001$), monocyte ROS resulted 2102.5 ± 517.5 vs 1253 ± 171 ($P < .001$), and granulocyte ROS were 2366.5 ± 595.4 vs 1751.0 ± 213.0 ($P < .001$). Significant increases plasma lipid peroxidation markers were found in NOA patients compared with controls (2.7 ± 0.8 vs 0.37 ± 0.2 nmol/mL, $P < .001$). Significant decreased TAC was evident in NOA compared with controls (13.4 ± 3.9 vs 3.0 ± 0.2 µmol/mL Trolox equivalents, $P < .001$). No significant differences were found in blood leukocyte subpopulations ROS production, plasma lipid peroxidation, and TAC comparing groups (positive vs negative sperm retrieval, $P > .05$).

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Conclusion: ROS production can be directly related to disorders of spermatogenesis, leading to severe conditions of male infertility, including azoospermia.

KEYWORDS
azoospermia, oxidative stress, oxygen radical absorbance capacity, reactive oxygen species, sperm retrieval

1 | INTRODUCTION

Infertility is a multifactorial disorder of the reproductive system, affecting 15% of couples in reproductive age.[1] The Male factor is responsible for 40%-50% of infertility cases.[2] Nowadays, about 1% of men and 10%-15% of the infertile male population may present azoospermia, as the complete absence of spermatozoa in the ejaculate[3] after assessment of centrifuged semen on at least two semen analysis.[4,5] Its etiology is unknown in at least 40% of cases, possibly because many of the genes that regulate spermatogenesis have not been yet identified.[6]

In non-obstructive azoospermia (NOA), sperm retrieval through testicular sperm extraction (TeSE) for in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) purpose represents for these men the main opportunity to father their biological child.[7,8]

Male infertility diagnosis is commonly based on standard semen analysis which, however, cannot clearly distinguish fertile from infertile populations.[9] Several causes of male infertility have been recognized, nevertheless, a large proportion of infertile males does not receive a clear diagnosis, and thus, they are reported as idiopathic or unexplained.[10]

Oxidative stress (OS) is one of the most prevalent causes of sperm damage, through the toxic effects of endogenously generated hydrogen peroxide (H_2O_2), superoxide anion ($O_{2\circ}^-$), and hydroxyl radicals (OH^-).[11] Indeed, OS is a condition associated with an increased rate of sperm damage due to cellular injury to sperm lipids, DNA, and proteins.[12] When normal sperm cells defense mechanisms fail, an imbalance between reactive oxygen species (ROS) production and total antioxidant capacity (TAC) occurs. This process is responsible for an adaptive response consisting of the induction of antioxidant response and, following antioxidant depletion, in cellular injury and dysfunction.[13] A physiological amount of ROS is important for motility promotion, acrosome reaction, capacitation, and further cell signaling pathways, including nuclear condensation as well as gamete fusion.[14] Otherwise, pathological elevated ROS levels generate a cascade of events leading to plasma membrane lipid peroxidation. The major ROS sources are represented by sperm mitochondria[15] morphologically abnormal spermatozoa and activated leukocytes in the seminal plasma[16,] in response to a wide range of infection/inflammation-related stimuli in the male genital tract.[17]

However, as described in previous studies,[18] peripheral leukocytes represent a feasible model for studying the pathophysiology of oxidative stress-mediated homeostasis variations, which can be responsible for cell dysfunction and cell injury. Leukocytes, indeed, reflect the condition of the whole organism and thus represent a valuable model to study systemic oxidative stress-related disorders.

A possible approach to improve fertility potential could be to reduce ROS levels in seminal plasma by oral supplementation with antioxidants, in order to increase the chance of natural conception, thereby improving the assisted reproductive technologies (ARTs) outcomes.[19]

On the understanding that higher ROS levels are shown in asthenozoospermic men, ROS and TAC estimations have been never assessed in azoospermic patients.

Given this background, the aim of this study was to evaluate the redox status, by measurements of blood leukocytes intracellular ROS levels, plasma lipid peroxidation, and plasma TAC in patients with NOA undergoing TeSE, compared with healthy normozoospermic subjects.

2 | MATERIAL AND METHODS

2.1 | Study population

This prospective observational monocentric study included 39 infertile couples who attended the ARTs Centre of Careggi Hospital, University of Florence, for IVF procedures, from May 2018 to March 2019. All patients were divided into two groups: Group 1 comprised infertile men with NOA, and Group 2 included age-matched normozoospermic men belonging to couples with female infertility tubal factor who achieved pregnancy using IVF or ICSI. NOA was defined as no sperm in the ejaculate due to the failure of spermatogenesis, whose etiology can be related to either intrinsic testicular impairment or inadequate gonadotropin production. The exclusion criteria were leukocytospermia (defined as leukocyte concentration greater than 1×10^6/mL), history of smoking, excessive alcohol consumption (≥15 drinks per week), endocrine disorders, drug intake, and those with varicocele. Also, men with significant comorbidities, including cancer, diabetes, obesity, autoimmune disease, gastrointestinal disease, kidney disease, and lung disease, were excluded from the study.

2.2 | Male assessment

Baseline parameters of all patients were collected from our clinical reports, including age, height, and weight with body mass index (BMI) count, previous diseases, or surgery. All the participants underwent semen analysis at baseline visit. The Hormonal panel, including

follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), thyroid-stimulating hormone (TSH), and prolactin (PRL), was required. Normal levels of FSH, LH, and TT were considered, respectively, 1.5-8.0 IU/L, 1.8-12 IU/L, and 2.7-18 ng/mL. PRL levels were considered normal between 3.0 and 18 ng/mL and TSH between 0.3 and 5.5 mIU/L. The Genetic evaluation was performed, including the karyotype with the examination of microdeletions for chromosome Y and mutation of cystic fibrosis transmembrane conductance regulator (CFTR) genes. All men performed a urine analysis and urethral swab to detect urinary tract infections. The serological tests for diagnosis of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, Treponema pallidum haemagglutination (TPHA), and rapid plasma reagin (RPR) tests were done. All infertile patients underwent genital examination and scrotal Doppler ultrasounds to establish testicular volume and to exclude the presence of epididymis head or tail dilatation, unilateral, or bilateral absence of vas deferens and varicocele. Testicular volume was considered normal when it was ≥ 18 mL.[20] Transrectal ultrasounds were performed to rule out the presence of prostate median cysts and of anomalies of the seminal vesicles suggestive of obstruction of the male genital tract.[21] For each patient who underwent TeSE, sperm retrieval (SR) data were recorded, including sperm concentration, sperm motility, and cryo biosystem straws preserved. Based on the main morphological pattern, testicular histology was categorized as follows: hypospermatogenesis (HYPO), showing a reduction in the number of normal spermatogenetic cells, maturation arrest (MA), characterized by the absence of later stages of spermatogenesis, Sertoli cell-only syndrome (SCOS) when tubules lacked germ cells.

2.3 | TeSE and sperm preparation technique

All NOA patients underwent multiple bilateral TeSE. Testis samples were harvested through 3 separate transverse tunical incisions, respectively, in the upper, lower, and median pole of the testis. According to the size of the testis, two samples with a minimum of 3 × 2 × 1 millimeters (6 mm^3) up to 8 × 5 × 3 millimeters (120 mm^3) were sharply excised from each tunical incision. When the testicular size was less than 8 mL, a single equatorial incision on the albuginea was performed and surgical loupes (6× magnification) were used. The testicle was then split open bluntly, and tubules were retrieved aiming to locate and collect the larger ones with an increased chance of harboring spermatozoa. Once harvested, the fragments were immediately handed over to the embryologist. Each testicular tissue sample was placed in a sterile Petri dish with a medium solution and then stretched between two sterile glass slides under stereomicroscopy. Subsequently, the suspension was directly observed under inverted microscopy at 200× for a first evaluation of the presence of spermatozoa. Finally, all the suspension was transferred to tubes and, after sedimentation of the solid tissue, the supernatant containing free sperm cells was processed by centrifugation at 1800 rpm for 8 min. The pellet was then re-suspended in about 300 µl of flushing medium. Only the supernatant with free spermatozoa was utilized for ART purposes. If retrieved, spermatozoa were cryopreserved for later use. If the embryologist observed more than 1-2 sperms/field (corresponding to an estimated concentration of 0.001 × 10^6 spermatozoa/mL), sperm retrieval was considered to be successful.

2.4 | Sample collection

Blood samples were collected from the antecubital vein, after at least 8 h of fasting. Blood samples were collected in Vacutainer tubes containing 0.109 mol/L buffered trisodium citrate (1:10) or EDTA (0.17 mol/L). After centrifugation (1500 g for 15 min at 4°C), aliquots of sodium citrate plasma were stored at −80°C for further analyses.

2.5 | Laboratory: blood leukocytes intracellular ROS levels

As we already described,[22] after collection, 100 µL of EDTA-anticoagulated blood samples were re-suspended in 2 mL of BD fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson Biosciences, San Jose, CA, USA), gently mixed and incubated at room temperature in the dark for 15 min. Cells were then centrifuged (700xg for 7 minutes at 20°C), the supernatant was discarded, and cells were washed twice in PBS. Intracellular ROS levels were assayed by incubating cells with H_2DCF-DA (2.5 µM) (Invitrogen, Carlsbad, CA, USA) in RPMI medium without serum and phenol red for 30 min at 37°C. H_2DCF-DA is a chemically reduced form of fluorescein used as ROS indicator inside cells. Indeed, the 2',7'-dichlorofluorescein (DCF) fluorescent probe is particularly sensitive to several oxygen radical species as hydrogen peroxide, peroxynitrite, hydroxyl radicals, and also to superoxide anions. H_2DCF-DA represents the most versatile indicator of cellular oxidative stress and the gold standard for ROS measurement.[23] After labeling, cells were washed and re-suspended in PBS and immediately analyzed using FACS Canto flow cytometer (Becton-Dickinson, San Jose, CA, USA). The sample flow rate was adjusted to about 1000 cells/s. For a single analysis, the fluorescence properties of 20,000 cells were collected. The respective gates were defined using the distinctive forward-scatter and side-scatter properties of each cell population. Cell viability was controlled by flow cytometry with propidium iodide staining and was found to exceed 95%. Data were analyzed using BD FACS Diva software (Becton-Dickinson, San Jose, CA, USA).

2.6 | Laboratory: plasma lipid peroxidation estimation (thiobarbituric acid reactive substances assay, TBARS ASSAY)

Plasma thiobarbituric acid reactive substances (TBARS) levels were estimated using a TBARS Assay Kit (TBARS-Cayman) following the manufacturer's protocol. It is based on the thiobarbituric acid as an optimal reagent able to react with lipoperoxidation products (after 1 h at 95°C), leading to the formation of a chromophore adduct measured spectrofluorometrically with excitation at 530 nm and emission at 550 nm in a Microplate Fluorometer

(Biotek Synergy H1). Results were expressed in terms of malondialdehyde, MDA (nmol/mL).[22,24]

2.7 | Laboratory: plasma TAC assay (oxygen radical absorbance capacity assay, ORAC ASSAY)

The ORAC method (oxygen radical absorbance capacity) is based on the fluorescence decay of a fluorescent probe, fluorescein, consequent to its oxidation by free radical species (particularly peroxyl radical) generated after the thermal decomposition of azo compounds as 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). A fluorescein solution (6 nM) prepared daily from a 4 μM stock in 75 mM sodium phosphate buffer (pH 7.4) was used. Trolox (250 μM final concentration), a water-soluble analog of vitamin E, was used as a standard. 70 μl of each sample was pre-incubated for 30 min at 37°C in each well with 100 μL of fluorescein, and then, AAPH solution (19 mM final concentration) was added starting the reaction. Fluorescence was measured with excitation at 485 nm, and emission was measured at 537 nm in a Microplate Fluorometer (Biotek Synergy H1). Results were expressed as Trolox Equivalents (μM).[25]

2.8 | Ethics statement and statistical analysis

The study protocol was performed in accordance with the Declaration of Helsinki and approved by the Ethical Review Board of the University of Florence (reference no. 10709/2017). All participants gave written informed consent to collect their data before entering the study and to use their peripheral blood sample. For descriptive aspects, data are summarized as means ± SD. Differences between groups were assessed by the Student independent t-test or the Mann–Whitney U-test based on their normal or not-normal distribution, respectively (normality of variables' distribution was tested by the Kolmogorov–Smirnov test). Categorical variables are presented as rate and percentage and were tested with the chi-square test. $P < .05$ was accepted as statistically significant. All collected data were evaluated with Statistical Package for Statistical Sciences (SPSS, version 22.0), IBM, Chicago, IL, USA).

3 | RESULTS

Baseline patients' characteristics are reported in Table 1. Group 1 comprised 19 men with a clinical presentation of absolute azoospermia, while group 2 included 20 normozoospermic men. Screening for sexually transmitted diseases was negative in all patients. No genetic abnormalities and significant comorbidities were detected in all men with NOA. All normozoospermic men showed serum hormonal levels and testicular volume in the normal range.

Data of infertile patients (NOA) were collected as follows. Mean age was 37.6 ± 6.2 years. Mean body max index (BMI) was 24.6 ± 1.5. Mean duration of couple infertility was 22.8 ± 8.7 months. At baseline, mean FSH value was 18.6 ± 7.15 IU/L, mean LH value was 7.4 ± 3.2 IU/L, mean TT was 12.5 ± 3.9 nmol/L, mean TSH was 2.0 ± 1.1 mIU/L, and mean PRL was 10.5 ± 3.2 ng/mL. Mean right testicular volume was 9.9 ± 6.6 mL, and mean left testicular volume was 8.4 ± 4.5 mL. Successful SR with cryopreservation was found in 10/19 patients (overall SR rate: 52.6%). Mean sperm concentration was 0.0005 ± 0.0006 × 10^6/mL. Mean non-progressive (NP) motility was 0.4 ± 0.6%. The mean number of cryo biosystem straws collected was 1.9 ± 2.0. No post-operative complications occurred following TeSE. Histological findings detected HYPO in 10/19 (52.6%), MA in 5/19 (26.3%) and SCOS in 4/19 (21.0%)

TABLE 1 Baseline variables of NOA patients compared with controls (tot = 39)

Variable	NOA (n = 19)	Controls (n = 20)	*P* value
Age (years)	37.6 ± 6.2	37.2 ± 5.8	NS
BMI	24.6 ± 1.5	24.8 ± 1.1	NS
FSH (IU/L)	18.6 ± 7.15	5.5 ± 2.3	*<.05**
LH (IU/L)	7.4 ± 3.2	3.4 ± 1.5	NS
TT (nmol/L)	12.5 ± 3.9	13.2 ± 4.1	NS
TSH (mIU/L)	2.0 ± 1.1	2.4 ± 1.2	NS
PRL (ng/mL)	10.5 ± 3.2	11.5 ± 2.8	NS
Right TV (mL)	9.9 ± 6.6	20.1 ± 1.5	*<.05**
Left TV (mL)	8.4 ± 4.5	19.5 ± 1.1	*<.05**

Note: Variables are expressed as mean ± standard deviation.
*Statistical significance.

The evaluation of blood systemic redox status was performed in NOA and normozoospermic subjects by the assessment of intracellular ROS levels in blood leukocyte subpopulations of lymphocytes, monocytes, and granulocytes. As reported in Figure 1, NOA patients showed a significant increase in ROS levels in all three leukocyte fractions compared with healthy normozoospermic subjects ($P < .01$). In particular, mean lymphocyte ROS production resulted 967.0 ± 224.5 vs 728.0 ± 98.0 (NOA vs Controls, $P < .001$),mean monocyte ROS production was 2102.5 ± 517.5 vs 1253.0 ± 171.0 (NOA vs Controls, $P < .001$); and mean granulocyte ROS production resulted 2366.5 ± 595.4 vs 1751.0 ± 213.0 (NOA vs Controls, $P < .001$).

As shown in Figure 2, a significant increase in mean plasma lipid peroxidation markers in NOA patients compared with controls was found (2.75 ± 0.8 vs 0.37 ± 0.2 nmol/mL, respectively, $P < .001$). Moreover, a significant decrease in mean plasma total antioxidant capacity (TAC) was evident in NOA patients compared with controls (13.4 ± 3.9 vs 3.0 ± 0.2 μmol/mL, respectively, $P < .001$).

As shown in Table 2, higher ROS levels of lymphocytes, monocytes, and granulocytes subpopulations in azoospermic patients with successful SR using testicular open biopsy technique were found. However, no significant differences were found in blood leukocyte ROS production comparing groups (positive vs negative sperm retrieval, $P > .05$). Similarly, plasma lipid peroxidation and TAC values in patients with successful sperm retrieval resulted comparable to those of patients with negative sperm recovery ($P > .05$).

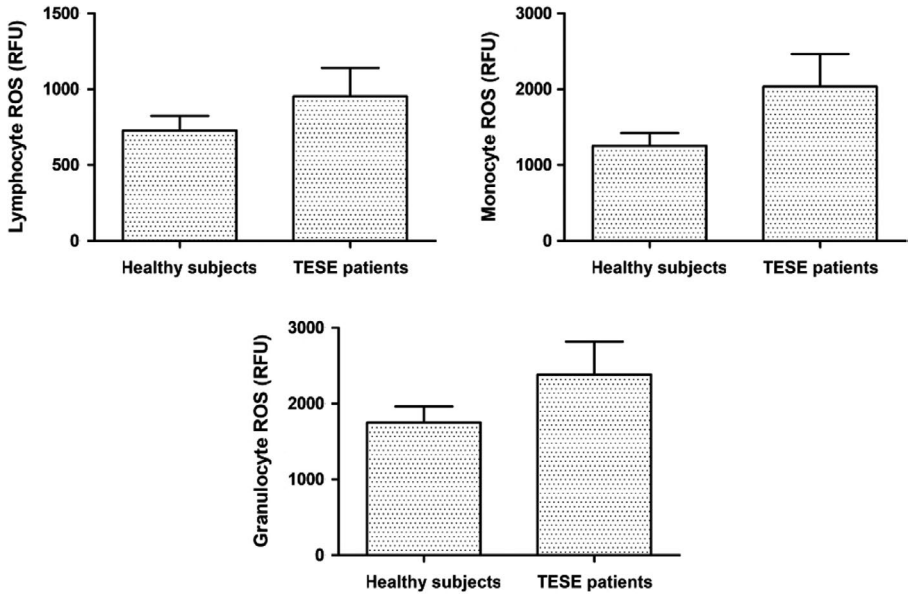

FIGURE 1 Comparison of blood lymphocyte, monocyte, and granulocyte ROS production between men with non-obstructive azoospermia (NOA) and controls

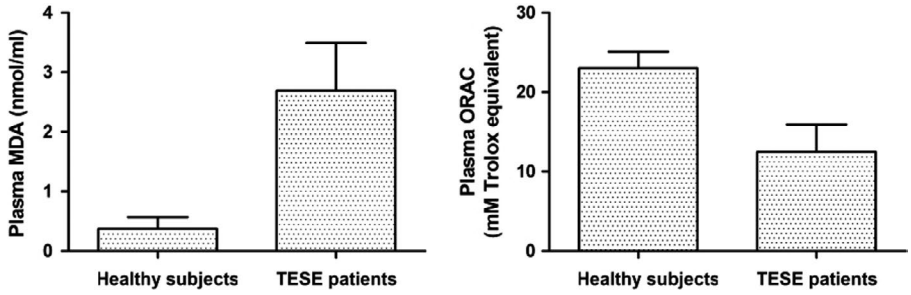

FIGURE 2 Plasma lipid peroxidation markers (MDA) and plasma total antioxidant capacity (TAC) in NOA patients compared to controls

TABLE 2 Blood leukocytes intracellular ROS levels, plasma lipid peroxidation, and TAC values in patients underwent TESE, comparing groups (positive and negative sperm retrieval)

Variable	Sperm retrieval + (n = 10)	Sperm retrieval – (n = 9)	*P* value
Lymphocyte ROS	966.7 ± 224.5	940.7 ± 229.9	NS
Monocyte ROS	2102.5 ± 517.5	1985.1 ± 468.9	NS
Granulocyte ROS	2366.5 ± 595.3	2274.3 ± 603.4	NS
Plasma lipid peroxidation	2.7 ± 0.8	2.8 ± 0.8	NS
TAC	13.4 ± 3.9	13.4 ± 4.1	NS

Note: Variables are expressed as mean ± standard deviation.

4 | DISCUSSION

The main result of this study is the presence of a marked redox imbalance, witnessed by significantly increased blood leukocyte ROS production and plasma oxidative stress markers in NOA patients compared with normozoospermic men.

Several previous studies showed that intracellular ROS overproduction is responsible for testicular damage and male reproductive disorders.[26-29] However, the evaluation of redox status, and in particular leukocyte ROS production, in the blood of patients consulting for infertility due to azoospermia has never been reported. Usually, physiological ROS levels are essential for normal spermatozoa functioning.[30] Otherwise, pathological ROS concentrations are commonly associated with sperm abnormalities, mainly due to ATP depletion and lipid peroxidation of the sperm membrane.[31-34] In line with these observations, signs of OS in human spermatozoa with a lower percentage of motility and morphology have been widely reported in literature.[28,35,36] Essentially, OS may display negative effects on sperm function,[37,38] due to the excess of plasma membrane polyunsaturated fatty acids which represent specific ROS targets.

Moreover, ROS also damage sperm nuclear DNA, displaying negative effects on sperm concentration and morphology and causing an increased risk of DNA fragmentation.[28] In this context, previous research reported that individuals with high leukocyte DNA damage have a high percentage of sperm DNA fragmentation, to use leukocyte DNA integrity as a possible tool to identify

patients at high risk, thus improving the reproductive outcomes before ICSI.[39]

Furthermore, it has been suggested that H_2O_2, which can diffuse across the membranes, inhibits the activity of enzymes such as glucose 6-phosphate dehydrogenase, thus leading to reduced cytoplasmic NADPH availability. As a result, oxidized glutathione accumulates, causing a decrease in global spermatozoa antioxidant capacity.[40] In line with these findings, our data showed significantly higher ROS levels in azoospermic men, as well as reduced TAC.

Additionally, it was recently demonstrated that mitochondrial ROS generation could trigger autophagy[41,] leading to the accumulation of damaged mitochondria and enhanced ROS production. All the above processes negatively affect blood–testis barrier (BTB) integrity, contributing to distorted spermatogenesis.[42,43,44]

On this basis, the primary objective of the present study was to evaluate redox balance in patients with azoospermia, in order to establish the potential role exerted by ROS in the genesis of testicular secretory injury, when NOA occurs.

Actually, our results lead us to believe that OS could be directly related to distorted spermatogenesis, until NOA condition. To effort our hypothesis, other authors addressed this issue. Indeed, previous studies showed that OS could reduce testicular volume, via Sertoli cell degeneration[45,46,] since Sertoli cells line up seminiferous tubules and make up to 90% of the testicular tissue.[47] In particular, systemic and/or local OS might be the cause of this negative relationship by reducing the volume and number of Sertoli cells via lipid peroxidation, DNA fragmentation, and apoptosis. This likely resulted in damage to tubules, causing testicular atrophy, reduction in motility, and DNA injury to mature sperm cells, with consequent negative effects on fertility and reproductive potential. Moreover, the reduction of Sertoli cell amount could have negative feedback on the hypothalamus leading to increased LH and FSH secretion. Indeed, OS negatively impacts Sertoli cell secretion of inhibin b that plays a key role in the hypothalamic–pituitary–gonadal axis, reducing FSH secretion via negative feedback on the pituitary gland. Consequently, the negative feedback mechanism resulted in increased FSH levels.[48] However, in our case series, LH and testosterone concentrations did not result significantly different between NOA patients and controls. The exact mechanism behind the negative correlation between OS and testosterone levels is not fully understood. Probably, it might also reflect a lower sensitivity of Leydig cells to oxidative stress. To confirm our hypothesis, several studies not identified any correlation between small testicular volume related to varicocoele and LH.[49,50] Indeed, any changes in Sertoli cell function and inhibin b secretion should not impact LH secretion.[18] However, further investigations are warranted to fully understand the mechanism behind the correlation between OS and LH/testosterone concentrations.

SR techniques, such as open testicular biopsy, represent the unique opportunity to allow NOA patients to be father of their biological child. However, in the case of secretory azoospermia, the likelihood of SR does not exceed 60%, because of intrinsic damage of spermatogenesis. In this context, several predictive factors, including testicular volume or hormone parameters, can be useful to optimize the better therapeutic approach and to counsel properly the infertile patients.[51] In particular, serum FSH concentration seems to be inversely related to the total number of testicular germ cells.[51] Similarly, the smaller testicular volume is associated with poorer possibility of SR, although there is no minimum limit of the size that predicts the presence of spermatozoa.[52]

However, there are no absolute defined prognostic factors or tests that can accurately predict the presence of spermatogenesis in azoospermic patients.[53]

Starting from this point, we investigated whether leukocyte ROS production and plasma oxidative stress markers could represent new systemic indicators/predictors of sperm retrieval. In this setting, an appropriate management with specific redox balancing treatments before TeSE could be fundamental to minimize oxidative insult thus optimizing the chance of sperm retrieval. Indeed, considering that cryopreservation, involving centrifugation, is a procedure associated with detrimental effects on sperm function due to increased lipid peroxidation and membrane permeability,[54,55,56] redox status homeostasis preservation before TeSE could be an advantage able to improve fertility outcomes.

However, in our case series, no significant differences were found in blood leukocyte ROS production between patients with successful SR and those with negative. Similarly, plasma lipid peroxidation and TAC values were comparable between groups. It follows that higher ROS levels are not significantly associated with poorer sperm retrieval possibilities. The small number of NOA patients enrolled, which represents the main limitation of this study, could explain this finding and we hope in future studies with larger cohort population to focus on this topic, in order to demonstrate whether the adherence to an oral administration with antioxidant compounds could increase sperm retrieval rates. Supplementing patients with specific antioxidants for a prescribed duration could help to improve their antioxidant capacity hence optimizing the fertilization capacity of suboptimal spermatozoa. Moreover, future researches should focus on which antioxidants, including glutathione, vitamin E, vitamin C, carnitines, coenzyme Q10, selenium, zinc, folic acid, and lycopene, could be more appropriate for maintaining redox homeostasis.

5 | CONCLUSION

The present study demonstrated that increased ROS production can be directly related to disorders of spermatogenesis, leading to severe conditions of male infertility, including azoospermia. Our results suggest that blood leukocyte ROS production is increased in NOA patients undergoing TeSE compared with normozoospermic men. However, future researches are needed to find predictors of sperm retrieval and to evaluate whether a specific oral antioxidant supplementation could reduce OS in these patients.

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None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

GC, MB, CF, MEC.: conceptualized and designed, drafted the article, and acquired the data. RF, RP, AC, PF, FB, AM, FR., and A.: acquired the data and analyzed and interpreted the data. AN, LC, MC, and SS: involved in critical revision. All of the co-authors interpreted the data and participated in finalizing the article. All of the co-authors approved the final version of the article.

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