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Chapter 6

Reactive Oxygen Species and Sperm Cells

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Abstract

Many cases of male factor infertility are idiopathic, but 30–40% of cases may have excessive levels of reactive oxygen species (ROS) in their semen. The origins of endogenous ROS are leukocytes and immature spermatozoa, and external causes are various. On the contrary, seminal plasma contains various antioxidants. Low levels of ROS are essential for the fertilization process, but excessive levels of ROS lead to oxidative stress and can have harmful effects such as lipid peroxidation of a membrane, sperm deoxyribonucleic acid fragmentation, and apoptosis on the fertile capacity. In order to evaluate oxidative stress appropriately, ROS is measured by the chemiluminescence method with neat semen and quantification of 8-OH-2′-deoxyguanosine and malondialdehyde in seminal plasma. Antioxidant potential is often measured using total antioxidant capacity (TAC) assay. The oxidation-reduction potential measured by a MiOXSYS analyzer is a novel, easier, quicker, and less expensive technology to measure oxidative stress. In order to minimize oxidative stress and improve clinical outcomes, sperm-sorting methods, lifestyle modifications, shortening the ejaculatory abstinence, and treatments such as oral antioxidants, varicocelectomy, and testicular sperm extraction are taken into account. As a future prospect, proteomics, metabolomics, and genomics are still developing areas that have the potential to discover new findings and highly sensitive biomarkers.

Keywords: reactive oxygen species, oxidative stress, lipid peroxidation, sperm DNA fragmentation, antioxidants, male infertility

1. Introduction

Infertility is defined as the inability to achieve pregnancy despite 1 year of regular, unprotected, and well-timed intercourse [1], and it is a global problem faced by 15% of couples. Approximately 50% of all cases presenting at infertility clinics are due to male factors [2]. However, the etiology of male factors is multifactorial and many cases are idiopathic [2]. Various studies have been

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conducted to elucidate the pathologies of idiopathic male infertility. Since Aitken and Clarkson first detected reactive oxygen species (ROS) in processed human ejaculate using the chemiluminescence method in 1987 [3], there have been many reports on the influence of oxidative stress on male fertile capacity. A low level of ROS is essential for the process of fertilization, such as capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion [4, 5]. On the contrary, a high level of ROS results in lipid peroxidation (LPO), deoxyribonucleic acid (DNA) damage, and induction of apoptosis [6], which has been reported to negatively affect sperm concentration [7, 8], motility [9–11], morphology [12, 13], and male fertile capacity [14]. Clinically, several studies reported that oxidative stress resulted in significantly lower fertilization rates, implantation failure, impaired embryonic development, recurrent pregnancy loss, lower live-birth rates, and poor assisted reproductive treatment (ART) outcomes [15–17]. It is considered that an understanding of the etiologies and influences of ROS on sperm, measuring oxidative stress appropriately, and treating based on the etiologies play an important role in improving the outcomes of male infertility.

2. Etiology of ROS in human semen

Oxidative stress in human semen develops as a result of an imbalance between ROS production and antioxidant capacity [18]. ROS are categorized as (1) free radicals, such as superoxide anion ($\cdot O_2^-$), hydroxyl radicals ($\cdot OH$), and peroxyl radicals ($\cdot RO_2$) and (2) nonradical species, such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HClO) [19]. Free radicals contain at least one unpaired valence electron that are highly reactive but have a short life span. Therefore, they become paired by depriving an unpaired electron from other compounds, which causes oxidation. Nonradical species, such as hydrogen peroxide itself, is not very reactive, but it generates hydroxyl radicals in the presence of metal ions in vivo [20].

However, it has become apparent in the past two decades that ROS at low levels function as signaling molecules to regulate biological and physiological processes [21]. ROS induce cyclic adenosine monophosphate in spermatozoa and elevate the level of tyrosine phosphorylation. Localization of tyrosine phosphorylation to the flagellum also leads to hyperactivation in the female genital tract. Moreover, tyrosine phosphorylation leads to binding the spermatozoon to the zona pellucida and is necessary for the acrosome reaction [4, 5]. On the contrary, oxidative stress caused by high levels of ROS has various adverse effects in a living body, such as cranial nerve disease (e.g., Alzheimer's disease and Parkinson's disease), arteriosclerosis, diabetes, and inflammatory bowel disease (e.g., ulcerative colitis and Crohn's disease) [22–25]. Similarly, ROS in semen function as an essential second messenger in the fertilization process at low levels, including capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion [4, 5]. Conversely, high levels of ROS in semen have been reported to be present in 25–40% of infertile men [9, 15, 26, 27]. Oxidative stress in semen is well known to cause LPO of the sperm membrane, which leads to a loss of fluidity, sperm DNA fragmentation, and apoptosis.

The principal sources of endogenous ROS in semen are seminal leukocytes [3, 28] and immature spermatozoa with an abnormal head morphology and cytoplasmic retention [29, 30]. When inflammation and infection occur in the male genital tract, chemotaxis and activation of leukocytes are stimulated, and they destroy pathogens by activating the myeloperoxidase system

[26], which produces ROS. On the contrary, in morphologically normal spermatozoa, cytoplasm deposits in the midpiece are extruded to allow cell elongation and condensation to occur during spermiogenesis, and the cytoplasm deposits contain large amounts of the glucose-6-phosphate dehydrogenase enzyme that produces nicotinamide adenine dinucleotide phosphate (NADPH). As a result, ROS is generated from NADPH via an intramembrane-located NADPH oxidase (NOX) [31]. So, immature spermatozoa are characterized by large amounts of cytoplasm that are expected to produce higher levels of ROS. It is important to determine the source of ROS in semen because the clinical implications of infiltrating leukocytes are quite different from those of pathological conditions in which the immature spermatozoa themselves are the source of ROS [32].

Myeloperoxidase staining (the Endz test) is useful to differentiate leukocytes (neutrophils and macrophages) from germinal cells [33]. Peroxidase-positive leukocytes in semen are identified for their capacity to generate high levels of ROS, contributed largely by the prostate and seminal vesicles [34]. These activated leukocytes can produce 100-fold higher amounts of ROS than nonactivated leukocytes because they increase NADPH production via the hexose monophosphate shunt [35].

Nitroblue tetrazolium (NBT) staining is used to detect ROS generation by immature spermatozoa [36]. NBT is a yellow water-soluble nitro-substituted aromatic tetrazolium compound that reacts with cellular superoxide ions to form a formazan derivative that can be monitored spectrophotometrically. Oxidation in the cytoplasm helps transfer electrons from NADPH to NBT and reduces NBT into formazan. The principle of this test is based on the conversion of NBT into blue-pigmented diformazan after interacting with superoxide. The concentration of diformazan is correlated with intracellular ROS concentration [37]. This NBT staining is a cost-effective and easy-to-use method that can predict ROS levels and simultaneously detect the source of ROS generation.

3. External causes of potential oxidative stress

External causes of ROS are shown in **Table 1**. They are classified roughly into lifestyle, environment, infection, autoimmune, testicular, idiopathic, iatrogenic, and chronic disease factors. Especially, tobacco contains more than 400 kinds of constituents, including nicotine, tar, carbonic monoxide, polycyclic aromatic hydrocarbons, and heavy metals, so the toxicological mechanism of smoking is complicated. Especially, nicotine is oxidative and can induce double-strand DNA breaks in sperm DNA in vivo [38]. It is reported that smokers had a 48% increase in seminal leukocyte levels and a 107% increase in seminal ROS levels compared with nonsmokers [39]. The sperm DNA fragmentation index (DFI) is also increased in infertile smokers compared with infertile nonsmokers (37.66% vs. 14.51%, $P < 0.001$) [40]. Furthermore, natural antioxidants such as vitamins C and E in seminal plasma were decreased in smokers, which indicates a reduced protection against oxidative stress [41].

Scrotal. hyperthermia may result from wearing close-fitting underwear, sauna use, longtime bathing, and cycling [42–45]. The position of the scrotum acts to maintain the temperature of the testes (34–35°C) lower than that of the body (36–37°C) [46]. An elevated scrotum temperature may negatively but reversibly affect spermatogenesis and oxidative stress. Rao et al. concluded that intermittent heat exposure could more seriously damage spermatogenesis than consecutive heat exposure [45]. Oxidative stress may participate in the suppression of spermatogenesis.

Lifestyle	Smoking
	Obesity
	Alcohol abuse
	Aging
Environmental	Pollution
	Heavy metals
	Heat
	Mobile phone radiation
	Phthalate
Infection	Genitourinary tract
Testicular	Varicocele
	Testicular torsion
Iatrogenic	Centrifugation
	Cryopreservation
	Drug
Others	

Table 1. External cause of oxidative stress.

Obesity also provokes oxidative stress because proinflammatory cytokines are released from adipose tissues, which leads to an increase in leukocytes [47]. Moreover, in numerous studies, a positive correlation between body mass index and DFI has been reported [48].

A clinical varicocele is almost exclusively left-sided and is a pampiniform plexus of the spermatic cord that forms a tangle of distended blood vessels in the scrotum. It has an incidence of approximately 15% in the general male population and 30–40% in men with primary infertility and 75% with secondary infertility [49]. A varicocele is considered to be the most common surgically correctable cause of male infertility [50]. Oxidative stress is considered to be the main factor contributing to infertility in men with a varicocele, to which the testis responds by way of high scrotal temperature, testicular hypoxia, adrenal metabolite backflow, or production of vasodilators such as nitric oxide [51]. The dominant mechanism is that impairment of protamination and chromatin compaction in sperm increases the susceptibility of affected cells to oxidative stress causing defective spermiogenesis [52]. Most studies reported that the level of seminal ROS in men with clinical varicoceles was higher than control subjects [53]. Moreover, the antioxidant level in both seminal plasma and blood was reduced in patients with a varicocele [54].

4. Lipid peroxidation

Elevated ROS production causes peroxidation of polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid, which has six double bonds per molecule in the sperm cell

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membrane [55]. This LPO of the sperm cell membrane causes a loss of membrane fluidity and integrity that are required for sperm-oocyte fusion [56]. LPO has two phases: the first phase is "initiation," which is the abstraction of a hydrogen atom from an unsaturated fatty acid, and the second phase is "propagation," which is the formation of a lipid alkyl radical followed by its rapid reaction with oxygen to form a lipid peroxyl radical [57]. A peroxyl radical can remove a hydrogen atom from an unsaturated fatty acid to produce a lipid radical and lipid hydroperoxide [58].

The products of LPO are malondialdehyde (MDA), conjugated dienes, and secondary peroxidation products such as saturated and unsaturated aldehydes, ketones, oxo- and hydroxyl acids, and saturated and unsaturated hydrocarbons (e.g., ethane and pentane). The methods used to detect and measure LPO include the spectrophotometric thiobarbituric acid (TBA) test, a fatty acid analysis by high-performance liquid chromatography, and an oxygen electrode. TBA test has been frequently used as an indicator of the peroxidation of PUFAs [55, 59]. Several studies have shown that LPO has detrimental effects on sperm concentration, motility, and morphology and is associated with poor sperm quality [60].

5. Sperm DNA fragmentation

Excessive amounts of ROS can damage sperm DNA directly or indirectly through the activation of sperm caspases and endonucleases. DNA fragmentation occurs after spermiation during comigration of mature and immature sperms from the seminiferous tubules to the cauda epididymis by ROS exposure, resulting in the formation of 8-OH-guanine and 8-OH-2′-deoxyguanosine (8-OHdG) [61].

DNA fragmentation can occur in single-strand (ss-) DNA and double-strand (ds-) DNA. The ss-DNA damage can be repaired by the human oocyte and embryo, although the repair ability decreases with advanced maternal age [62]. However, spermatozoa with ds-DNA damage fall into apoptosis [63]. The presence of unrepaired DNA damage above the critical threshold in an embryo has an adverse effect on embryo development and implantation, which has been characterized as the "late paternal effect." [64] On day 2–3 of human embryo development (between the four-cell and eight-cell stage), as embryonic genome activation begins, embryo development switches from being dependent on the maternal factor to being dependent on the embryo's own genomes [65]. Therefore, sperm with DNA damage has a detrimental effect on the rate of blastulation, implantation, and pregnancy. Several studies have investigated the relationship between ART outcome and high DNA damage in sperms. The meta-analysis of Zhao et al. showed that sperm DNA damage was significantly associated with pregnancy [combined relative risk (RR): 0.81; 95% confidence interval (CI): 0.70–0.95; $P = 0.008$] and miscarriage (combined RR: 2.28; 95% CI: 1.55–3.35; $P < 0.0001$) [66].

6. Antioxidants in human semen

All human ejaculate contains intra- and extracellular antioxidants in the seminal plasma. They are categorized as enzymatic and nonenzymatic antioxidants (**Table 2**) [67]. Enzymatic

Enzymatic antioxidants	Superoxide dismutase
	Glutathione peroxidase
	Catalase
Nonenzymatic antioxidants	Ascorbic acid (vitamin C)
	Alpha-tocopherol (vitamin E)
	Urate
	Coenzyme Q10
	L-Carnitine
	Melatonin
	Myo-inositol
	Lactoferrin
	Astaxanthin

Table 2. Antioxidants in human semen.

antioxidants contain superoxide dismutase (SOD), catalase, and glutathione peroxidase. Nonenzymatic antioxidants contain ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), urate, melatonin, transferrin, carnitine, and lactoferrin [67–69]. These antioxidants function as scavengers of ROS by self-protection mechanisms. A total antioxidant capacity (TAC) score has often been used to measure the total nonenzymatic antioxidant capacity in seminal plasma [69]. Seminal TAC can be measured as the total available antioxidants in the seminal plasma, while measuring by specific antioxidant assays is expensive, cumbersome to perform, and provides limited information about the assessed antioxidants [70]. The principle of this assay is based on the ability of all antioxidants in the seminal plasma specimen to inhibit the oxidation of 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) to $ABTS^{+}$, resulting in a change of absorbance at 750 nm to a degree that is proportional to their concentration. The capacity of the antioxidants present in the sample to prevent ABTS oxidation was compared with that of standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble tocopherol analog. The results are reported as micromoles of a Trolox equivalent. The total antioxidant concentration of each sample was calculated using the equation obtained from the linear regression of the standard curve by substituting the average absorbance values from each sample into the equation [71]:

$$\text{antioxidant } (\mu\text{M}) = \left[(\text{unknown average absorbance–Y} - \text{intercept})/\text{slope}\right] \times \text{dilution} \times 1000. \quad (1)$$

Past studies showed that low levels of seminal TAC were related to male infertility [72]. Mahfouz et al. reported the best cutoff value of seminal plasma TAC level was 1420 μM with high sensitivity and specificity [73]. However, TAC levels are not routinely measured as a standard infertility evaluation because TAC assay is cumbersome and employs costly equipment and skills. To measure more precise oxidative stress, the ROS-TAC score described below is more useful than TAC alone [12].

7. Measurement of ROS in human semen

ROS measurements can be determined by direct or indirect assays (**Table 3**). Direct assays measure the oxidation levels of the sperm cell membrane. Indirect assays measure the detrimental effects of oxidative stress, such as sperm DNA damage or LPO levels [74]. The chemiluminescence method is a direct assay that is commonly used to measure seminal ROS. Takeshima et al. [9] and Yumura et al. [11] reported that chemiluminescence was recorded using a computer-driven luminometer after the addition of 40 μL of 100 mmol/L 5-amino-2,3-dihydro 1,4-phtalazinedione (luminol) to 500 μL of unwashed semen. The Luminometer 1251™ (LKB Wallac, Turku, Finland) was used to measure the ROS level of unprocessed semen according to the previously reported method. When the peak level was ≥0.1 mV/s, ROS formation was considered positive. The integral level of ROS production in the present study was calculated by subtraction of the area under the baseline from total chemiluminescence values between 0 and 30 min after the addition of luminol to unwashed semen and expressed as mV/s/30 min/10^6 spermatozoa (**Figure 1**) [9]. The Monolight 3010™ Luminometer (BD Biosciences Pharmingen, Ltd., San Diego, CA, USA) was similarly used to measure ROS levels. The subtraction of the integrated chemiluminescence between 0 and 200 s before and after the addition of luminor to the sample was measured. The calculated chemiluminescence value was expressed as relative light units (RLU)/200 s/10^6 spermatozoa. The chemiluminescence ROS level threshold was defined as 4332.4 RLU/200 s [11]. A variety of luminometers are available. Single- and double-tube luminometers are sensitive and inexpensive but can only measure one or two specimens at one time, so it is not suitable for a center where many specimens are handled [75].

As described above, NBT assay is a cost-effective and user-friendly direct assay. The advantage of this assay is that it is can evaluate the ROS level and its potential source (such as spermatozoa or leukocytes) using a light microscope. The concentration of diformazan is correlated with the intracellular ROS concentration [36, 37].

MDA is an end product of LPO, and it represents the level of LPO. In a TBA assay, sperm MDA concentration is measured using spectrophotometry [76]. Sperm MDA levels are positively correlated with seminal ROS in infertile men [76].

8-Hydroxy-2-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base and is also used as a sensitive marker of oxidative DNA damage caused by ROS in human sperm [77]. 8-OHdG in seminal plasma can be measured by the enzyme-linked immunosorbent assay, and 8-OHdG

Direct assays	Indirect assays
Chemiluminescence	Myeloperoxidase (Endz) test
Nitroblue tetrazolium test	Lipid oxidation level
Flow cytometry	Oxidation reduction potential
Electron spin resonance	Total antioxidant assay

Table 3. Direct and indirect semen assays of ROS.

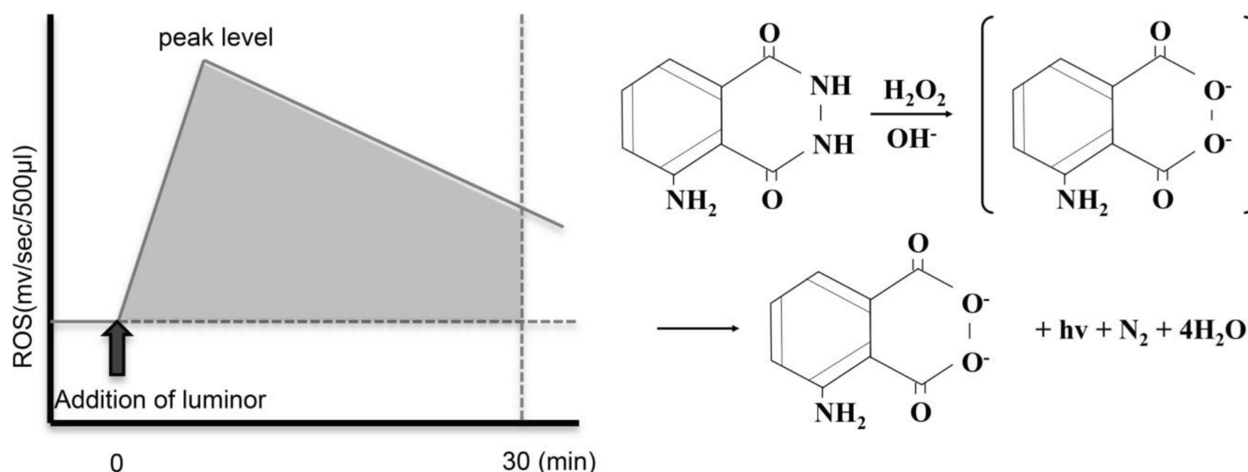

Figure 1. ROS measurement by chemiluminescence assay.

in testicular tissue can be quantified by immunohistochemical staining using an anti-8-OHdG monoclonal antibody as a primary antibody [78].

As described above, seminal TAC can be measured as the total available antioxidants in the seminal plasma [71]. But instead of ROS or the TAC score alone, an ROS-TAC score is thought to be a better predictor of oxidative stress [79]. The ROS-TAC score is a parameter derived from the ratio of ROS concentrations in washed sperm suspensions and TAC in seminal plasma using a principal component analysis to obtain a standard index of oxidative stress. A cutoff value of 30 is determined as the lower limit of a normal range ROS-TAC score. Patients with scores <30 are thought to be at risk of infertility [80]. However, a TAC assay is not carried out routinely because it is cumbersome and requires the use of costly equipment and technical skills [12].

Instead, the oxidation-reduction potential (ORP) is a direct measurement of oxidative stress by the MiOXSYS system, which is a novel, easier, quicker, and less expensive technology to measure the transfer of electrons from reductants to oxidants in human semen [81, 82]. Past studies have successfully evaluated ORP in the blood of patients with cerebral vascular disease, heart disease, and metabolic syndrome. In recent years, ORP measurements in semen have been carried out [81, 82]. Using a MiOXSYS Analyzer (Aytu BioScience Inc., Englewood, CO), ORP values were calculated and expressed as mV/10^6 spermatozoa/mL. A higher ORP level indicated an imbalance in the activity of all available oxidants relative to all available antioxidants in the ejaculate and indicates a state of oxidative stress. Agarwal et al. confirmed the cutoff level as 1.36 mV/10^6 spermatozoa/mL [83]. This MiOXSYS system has shown promise as a diagnostic tool in the evaluation of male infertility.

8. Prevention and treatment

There are several types of sperm-sorting methods, lifestyle modifications, and treatment strategies that can be used to minimize the detrimental effects of oxidative stress on reproductive function.

8.1. Sperm-sorting methods

Approaches of sperm selection that overcome production of oxidative stress and remove sperm with DNA damage include density gradient centrifugation (DGC) [84], electrophoretic separation [85], intracytoplasmic morphologically selected sperm injection (IMSI) [86], hyaluronic acid binding assay [87], and annexin-V magnetic activated cell separation (MACS) [88]. Especially, DGC can separate motile spermatozoa from immotile spermatozoa, leukocytes, cell debris, and toxic ROS prior to ART. Takeshima et al. reported that DGC could newly generate ROS, but ROS were pooled in the upper layer. Therefore, DGC can select mature spermatozoa without enhancing oxidative stress [84].

8.2. Lifestyle modification

External causes of ROS generation are shown in **Table 1**. To minimize exogenous ROS generation, cessation of smoking [89], weight loss through diet education and moderate exercise [90], and decreasing the opportunity of exposure to phthalate [91] are useful preventive measures. It is also well established that alcohol abuse [92], an elevated temperature around the scrotum [44, 45], and exposure to toxins such as heavy metals and organic solvents [93] lead to an increase in oxidative stress and can have harmful effects on one's fertile capacity. Avoiding cycling with tight pants, avoiding taking long hot water baths and saunas, and avoiding using a laptop on closed legs will also help to minimize oxidative stress [42–45]. Further, using protective equipment at work places that reduces the exposure to chemicals and vapors leading to oxidative stress is also an effective preventive way to minimize oxidative stress. Because mobile phone radiation increases ROS production and decreases antioxidant activities [94], storing a mobile phone somewhere other than a trouser pocket is a useful measure for minimizing oxidative stress [95].

8.3. Shorter period of ejaculatory abstinence

As mentioned above, sperm cells in the cauda of the epididymis and vas deferens may be subject to a harmful seminal microenvironment of oxidative stress before or after ejaculation. Therefore, increasing the ejaculation frequency may reduce spermatozoal exposure to toxic ROS, thereby improving sperm motile function. Several studies showed that a shorter period of ejaculatory abstinence was associated with a higher seminal TAC and a lower sperm DFI. But, there was no significant difference in the degree of sperm membrane LPO when comparing the period of ejaculatory abstinence [96]. A shorter period of ejaculatory abstinence may improve sperm quality and DNA integrity by protecting sperm from ROS damage.

8.4. Oral antioxidant therapy

An oral antioxidant treatment has been reported to reduce sperm damage and improve intracytoplasmic sperm injection (ICSI) outcomes in patients with oxidative stress and sperm DNA damage. According to systematic reviews in the Cochrane database [97], 48 randomized controlled trials (RCTs) that compared single and combined antioxidants with a placebo

in a population of 4179 infertile men were reviewed. The results showed that antioxidants might have increased clinical pregnancy rates [odds ratios (OR): 3.43, $P < 0.0001$, 7 RCTs, 522 men] and live birth rates (OR: 4.21, $P < 0.0001$, 4 RCTs, 277 men), but these evidences were graded low. Representative oral antioxidant therapies are vitamin C alone (400–1000 mg/day) [98] and a combination of vitamins C and E [99, 100]. Vitamins C and E work synergistically, and many studies on the positive effect of combined antioxidants on reducing DNA fragmentation and increasing the clinical pregnancy rate have been reported. Zinc is an element essential for spermatogenesis and sperm DNA synthesis. It also prevents LPO and works as a component of SOD [101, 102]. Selenium is also an essential component of the glutathione peroxidase selenoproteins [103]. Several studies reported that the mixed antioxidants including zinc and selenium could decrease sperm DNA fragmentation and increase clinical pregnancy rates [104]. L-Carnitine and coenzyme Q10 are strong antioxidants that prevent LPO and sperm DNA fragmentation. Meta-analysis indicated that both elements improved the conventional sperm parameters [105, 106]. And as to L-Carnitine, erectile function was also improved [105].

8.5. Varicocele repair

As described above, current evidences suggest that oxidative stress and elevated levels of sperm DNA fragmentation are the main factors that contribute to infertility in men with a varicocele [52]. Moreover, current evidences also suggest that varicocele repair in men who have a clinically palpable varicocele with documented infertility significantly improves the male fertile capacity [50, 107]. Surgical options for varicocele repair include the traditional high retroperitoneal (Palomo) and inguinal (Ivanissevich) approach, laparoscopic high ligation, and microsurgical low ligation via an inguinal or subinguinal incision. Microsurgical low ligation of the spermatic vein by the subinguinal approach is considered the gold-standard technique for varicocele repair because of lower postoperative recurrence and complication rates (e.g., hydrocele, testicular atrophy, and wound pain) compared to other techniques [108]. Several studies indicate that a varicocele repair reduces oxidative stress in seminal plasma and ameliorates sperm DNA damage. Moreover, varicocele repair significantly increases antioxidant levels indirectly because of a decrease in exhaustion due to less ROS formation after the surgery [109].

8.6. Testicular sperm extraction

As mentioned above, spermatozoa in the ejaculate are affected by ROS in the process of ejaculation. The testes have substantial antioxidant systems, but once spermatozoa are released from the Sertoli cells and migrate from the seminiferous tubules to the epididymis, they become susceptible to oxidative stress [110]. DNA damage in testicular spermatozoa is threefold lower compared with ejaculated spermatozoa [111]. Testicular sperm extraction (TESE) is a method of surgically retrieving sperm from the testis in patients with azoospermia or cryptozoospermia. ICSI using testicular sperm has a higher implantation rate and pregnancy rate than that using ejaculated sperm [112, 113]. However, testicular sperm has a significantly higher aneuploidy rate than ejaculated sperm [111]. Therefore, this method should be carried out with limited indication of recurrent ART failure and severe oligozoospermia cases.

9. Future prospects

In recent years, with the development of proteomics technology, identification of proteins that can be used as biomarkers of diseases has been carried out [114, 115]. Identifying the proteins expressed in spermatozoa and seminal plasma in semen with high ROS levels can lead to the discovery of new biomarkers of idiopathic male infertility. The proteomic analysis uses one- or two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to quantify the protein in addition to a shotgun analysis by mass spectrometry to qualify the protein expressed in sperm or seminal plasma. The current proven overexpressed proteins in the ROS-positive group include glutamine synthetase (GLUL), heat shock 70 kDa protein 5 (HSPA5), histone cluster 1, H2ba (HIST1H2BA), and sperm acrosomal membrane protein 14 [115].

Metabolomics, the study of cellular metabolic products and genomics, the study of identifying genetic abnormalities are current progressive research areas [116]. These studies have a great potential for identifying highly sensitive biomarkers.

Conflict of interest

The authors report no declarations of interest.

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