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Review on the role of antioxidant supplementation against oxidative stress: a human and animal approach to male fertility

Revisão sobre o papel da suplementação antioxidante contra o estresse oxidativo: uma abordagem humana e animal sobre a fertilidade masculina

Revisión del papel de la suplementación con antioxidantes contra el estrés oxidativo: un enfoque humano y animal de la fertilidad masculina

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Abstract
Male infertility is one important factor among the multifactorial causes of couple infertility, being oxidative stress one of the main related sources. Sperm is a specialized cell extremely susceptible to stress. To understand and mitigate this event, many studies have used different antioxidants, orally or *in vitro* supplementation, trying to improve sperm quality and function. Considering the extensive available literature regarding approaches and attempts to solve male fertility issues, the aim of this review is evaluating the effects of antioxidant supplementation on sperm, in both humans and experimental models with animals. This review selected original data from PubMed. The keywords used were: antioxidant, sperm, male fertility, antioxidant supplementation, male infertility; and the term "rodents" was added to the descriptors “antioxidant” and “male fertility”. Only studies published in indexed journals, in English, between 2015 and 2019 were included. This review involves i) human sperm and ii) rodent sperm. For the human approach, the search retrieved 496 articles and 80 were included, among which 28 studies were of *in vitro* antioxidant supplementation, 19 involved oral antioxidant supplementation and the remaining 33 concerned quantification of oxidants and antioxidants already present in the seminal samples. For the rodent approach, 152 articles were retrieved and 52 were included: 3 of varicocele, 11 of diabetes, 10 of therapeutic drugs, 3 of physical exercise, 10 of environmental exposure and 3 of heat stress. The remaining studies involved oxidative stress status in experimental models. Antioxidants use for reproductive purposes is increasing in an attempt to achieve better gametes and embryos. Vitamins C, B and E, selenium and zinc are the most commonly used antioxidants, with remarkable evidences in improving pathophysiological seminal conditions.
Keywords: Human reproduction; Reactive oxygen species; Sperm; Spermatozoa; Rodent; ART.

Resumo
A infertilidade masculina é um fator importante entre as causas multifatoriais da infertilidade do casal, sendo o estresse oxidativo uma das principais fontes relacionadas. O espermatozoide é uma célula especializada extremamente suscetível ao estresse. Para entender e mitigar esse evento, muitos estudos têm usado diferentes antioxidantes, suplementando via oral ou *in vitro*, na tentativa de melhorar a qualidade e função desses gametas. Considerando a extensa literatura disponível sobre abordagens e tentativas de resolver questões de infertilidade masculina, o objetivo desta revisão é avaliar os efeitos da suplementação com antioxidantes sobre os espermatozoides, tanto em humanos quanto em modelos experimentais com animais. Esta revisão selecionou dados originais do PubMed. As palavras-chave utilizadas foram: antioxidant, sperm, male fertility, antioxidant supplementation, male infertility; e o termo “rodents” foi adicionado aos descritores “antioxidant” e “male fertility”. Apenas estudos publicados em revistas indexadas, em inglês, entre 2015 e 2019 foram incluídos. Esta revisão envolve i) espermatozoides humanos e ii) espermatozoides de roedores. Para a abordagem humana, a busca recuperou 496 artigos e 80 foram incluídos, entre os quais 28 estudos eram de suplementação antioxidante *in vitro*, 19 envolviam suplementação antioxidante oral e os 33

restantes diziam respeito à quantificação de oxidantes e antioxidantes já presentes nas amostras seminais. Para a abordagem animal, 152 artigos foram recuperados e 52 foram incluídos: 3 de varicocele, 11 de diabetes, 10 de drogas terapêuticas, 3 de exercícios físicos, 10 de exposição ambiental e 3 de estresse térmico. Os demais estudos envolveram o status do estresse oxidativo em modelos experimentais. O uso de antioxidantes em técnicas de reprodução assistida vem aumentando na tentativa de obter gametas e embriões melhores. Vitaminas C, B e E, selênio e zinco são os antioxidantes mais comumente usados, com notáveis evidências na melhoria das condições seminais fisiopatológicas.
Palavras-chave: Reprodução humana; Espécies reativas de oxigênio; Espermatozoides; Roedores; TRA.

Resumen
La infertilidad masculina es un factor importante entre las causas multifactoriales de infertilidad de pareja, siendo el estrés oxidativo una de las principales fuentes relacionadas. El esperma es una célula especializada que es extremadamente susceptible al estrés. Para comprender y mitigar este evento, muchos estudios han utilizado diferentes antioxidantes, suplementados por vía oral o in vitro, en un intento por mejorar la calidad y función de estos gametos. Considerando la extensa literatura disponible sobre enfoques e intentos para resolver problemas de infertilidad masculina, el objetivo de esta revisión es evaluar los efectos de la suplementación con antioxidantes en los espermatozoides, tanto en humanos como en modelos experimentales. Esta revisión seleccionó datos originales de PubMed. Las palabras clave utilizadas fueron: antioxidante, esperma, fertilidad masculina, suplementación con antioxidantes, infertilidad masculina; y se añadió el término "roedores" a los descriptores "antioxidante" y "fertilidad masculina". Solo se incluyeron estudios publicados en revistas indexadas, en inglés, entre los años de 2015 y 2019. Esta revisión incluye i) esperma humano y ii) esperma de roedor. Para el brazo humano, la búsqueda recuperó 496 artículos y se incluyeron 80, entre los cuales 28 estudios fueron suplementos de antioxidantes in vitro, 19 involucraron suplementos de antioxidantes orales y los 33 restantes se referían a la cuantificación de oxidantes y antioxidantes ya presentes en las muestras. Para el brazo de roedor, se recuperaron 152 artículos y se incluyeron 52: 3 sobre varicocele, 11 sobre diabetes, 10 sobre fármacos terapéuticos, 3 sobre ejercicio físico, 10 sobre exposición ambiental y 3 sobre estrés por calor. Los otros estudios involucraron estrés oxidativo en modelos experimentales. El uso de antioxidantes con fines reproductivos está aumentando en un intento por obtener mejores gametos y embriones. Las vitaminas C, B y E, el selenio y el zinc son los antioxidantes más comúnmente usados, con una evidencia notable para mejorar las condiciones seminales fisiopatológicas.
Palabras clave: Reproducción humana; Especies reactivas de oxígeno; Esperma; Roedores; TRA.

1. Introduction

It is estimated that the male factor is responsible for half of couple infertility cases (Smit et al., 2007). Couple infertility is defined as lack of success in getting pregnant after 12 months of sexual intercourse without contraceptives (WHO, 2010). The literature suggests that male infertility is a multifactorial phenomenon. One of the essential factors that should be considered is oxidative stress (OS), which is present in 30 - 80% of idiopathic infertile men (Bisht et al., 2017). OS arises from an oxidative imbalance between reactive oxygen species (ROS) and the antioxidants present in the sperm microenvironment (Gulum et al., 2017; Sikka, 2001). Indeed, physiological amounts of ROS are fundamental to many reproductive events. Among them, we highlight sperm hyperactivation, capacitation, acrosome reaction, and zona pellucida interaction (Banihani et al., 2018; Moretti et al., 2017).

Sperm is a highly specialized and differentiated cell, in which the plasmatic membrane is rich in polyunsaturated fatty acids, promoting higher fluidity (Aitken, 1994), and the cytoplasmic volume is reduced, leaving space to organelles responsible for fundamental cell functions (such as the acrosome and mitochondria). With this morphology, the sperm has an important decrease in cytoplasmic antioxidants, making it more susceptible to oxidative Burst, which frequently occurs due to mitochondrial hyperfunction (Aitken & Clarkson, 1988). It is important to note that oxidative stress may occur at the testicular level, post-testicular, and in the ejaculate (Ogórek et al., 2017; Wagner et al., 1994), complexifying the study of the oxidative event. Understanding specific mechanisms are fundamental to develop precise and targeted antioxidant therapies, especially given the vast literature regarding attempts to promote oxidative balance.

Antioxidant therapy has been proposed as one possible way to minimize oxidative damage in the forms of higher sperm DNA fragmentation, lower mitochondria activity and acrosome integrity (Adami et al., 2018; J. Liu et al., 2016;

Martínez-Soto et al., 2016). Different approaches have been used in this area, for example, oral supplementation, *in vitro* supplementation, supplementation in cryopreservation or in animal models (Amidi et al., 2016; Donnelly et al., 1999; Panner Selvam et al., 2018; Raad et al., 2019). Moreover, the research of different methods indeed provides essential information to meet the demands of both research and medical practices through the development of biotechnologies. Assisted reproduction techniques (ART) and embryos and/or gametes manipulation seek higher rates of implantation, recovery, pregnancy and live births (Adami et al., 2019; Gual-Frau et al., 2015; Navas et al., 2017; Rago et al., 2017; Salian et al., 2019; Tesarik et al., 2004; Vieira et al., 2018); in this context, understanding the antioxidant function makes an essential contribution.

Some of the most critical factors influencing seminal quality improvement by antioxidant supplementation are the antioxidant used, *in vitro* incubation time, ideal antioxidant concentration, and whether co-supplementation with two or more substances was performed (Dias et al., 2015; Giacone et al., 2017; Gvozdjáková et al., 2015; Lv et al., 2018). We aim to identify the effects of using oral or *in vitro* antioxidants supplementation on human and rodent sperm characteristics through a literature review.

2. Methodology

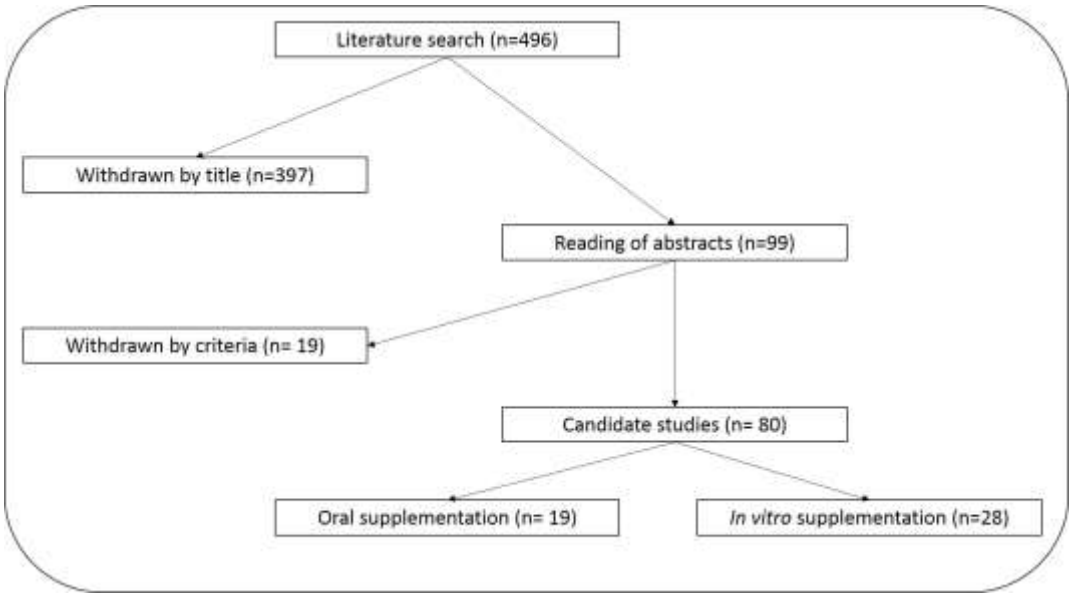
This review was performed in PubMed (MEDLINE) database because it had the largest number of studies in this field. The search strategy was developed using the following keywords: “antioxidant”, “sperm”, “male fertility”, and “antioxidant supplementation”. The terms "rodents" and “NOT review” were used together with keywords “antioxidant” and “male fertility” to filter studies in experimental models. To be included, studies should be published in indexed journals between 2015 and 2019, and in the English language.

The search was conducted between April and August of 2019; two authors (LNGA and VLMJ) performed the selection of studies independently. Results were divided into two categories: i) humans and ii) rodents. The first section covers human studies with oral and *in vitro* supplementation (when the antioxidant is supplemented into human semen sample after the ejaculate) to assess possible improvement in pre-established conditions. For this section, we excluded studies based on cryopreservation, with animal sperm, human seminal plasma, urine analysis, hormone analysis, genetic analysis, and assessing the effect of exposure to aggressors. We didactically divided the second session according to the condition studied since animal models are usually used to research conditions that lead to oxidative stress, verifying its impacts on seminal characteristics that could cause male infertility. In this section, we filtered abstracts that didn’t match the descriptors and didn’t present any seminal or sperm evaluation. Finally, the research and the manuscript were checked by all authors independently.

3. Results

For the human section, the search strategy retrieved 496 papers. Applying the exclusion criteria, 99 articles were selected, and after a second verification on study types, 80 were included. Of these, 28 used *in vitro* antioxidant supplementations, 19 were related to oral antioxidant supplementations, 22 involved studies which made oxidants and antioxidants quantifications already present in the human seminal samples after the ejaculate (Figure 1), and the remaining studies (11) were used on the way to introduce the “Redox balance in human sperm” section. All studies of this section can be found in Tables 1 and 2.

Figure 1: Flowchart representing the search and selection of studies in humans.



Source: Authors.

Table 1. Articles that used oral antioxidant supplementation.

Oral Antioxidantt	***Dose***	***Status***	***Findings***	***Reference***
Vitamin C	250 mg (daily)	Men operated on varicocele	It can be used as an adjuvant in the treatment after varicocelectomy to improve sperm parameters	Cyrus *et al* 2015
	250 mg (daily)	Males with unknown fertility	Inverse correlation between C vitamin levels and myeloperoxidase concentration in seminal plasma	Pullar *et al* 2017
	1g	Groups of normozospermic men based on BMI	Improvement in sperm concentration and motility	Bahare Rafiee *et al* 2016
Vitamin E	600mg	Men operated by inguinal varicocelectomy	Improvement in seminal parameters without statistical differences comparing with control group	Ener *et al* 2015
	400mg	Oligoasthenozospermic men	Improvement in sperm concentration and motility	ElSheikh *et al* 2015
Alpha lipoic acid	600mg	Asthenozospermic men	Improvement in total antioxidant capacity and in total count, concentration and sperm motility	Haghighian *et al* 2015
Tamoxifen	10mg	Oligoasthenozospermic men	Improvement in mitochondrial membrane potential and antioxidant enzymes	Li Guo *et al* 2015
Zinc	440mg	Asthenozospermic men	Normalization on activity of thiol-related enzymes in infertile patients	Alsalman *et al* 2017
Lycopene	30mg	Oligoasthenozospermic men	Decrease in seminal plasma white blood cells and increase in lycopene plasmatic concentration	Yu Yamamoto *et al* 2017
N-acetyl-cysteine	600mg	Asthenoteratozospermic men	Decrease in abnormal cells, DNA fragmentation and in protamines deficiency	Rahil Jannatifar *et al* 2019
Docosahexaenoic acid	1.5g	Men who were undergoing evaluation for infertility	Reduction of DNA damages	Martínez-Soto *et al* 2016
Curcumin	80mg	Oligoasthenozospermic men	Improvement in total antioxidant capacity, malondialdehyde, C-reactive protein, and tumor necrosis factor	Fatemeh Alizadeh *et al* 2017
Probiotics	*L. rhamnosus* and *B. longum* 10^9 cfu/day	Asthenozospermic men	Decrease of H_2O_2 intracellular levels	Valcarce *et al* 2017

BMI: Body Mass Index; Normozoospermic: normal seminal sample according WHO 2010; Varicocelectomy: surgery to repair varicocele condition; Oligoasthenozospermic: low sperm concentration and low sperm motility according WHO 2010; Asthenoteratozospermic: low sperm motility and low sperm normal morphology according WHO 2010; Asthenozospermic: low sperm motility according WHO 2010; WHO: World Health Organization. Source: Authors.

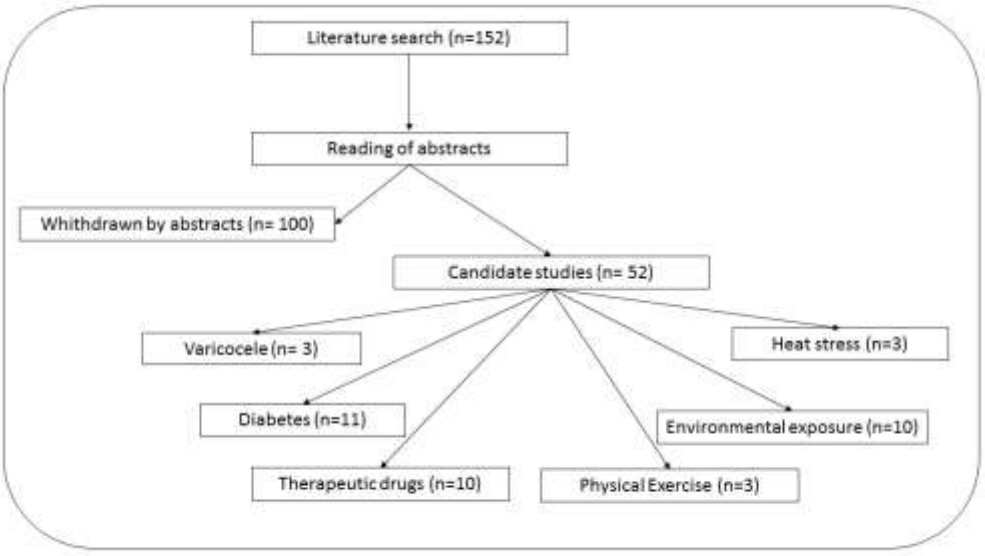
Table 2. Articles that used *in vitro* antioxidant supplementation.

In vitro Antioxidant	*Dose*	*Status*	*Findings*	*Reference*
Vitamin B	10nM	Normozospermic and asthenozospermic men	Increase in total motility	Salian *et al* 2018
Vitamin E	40uM	Sample challenged by H_2O_2	Improvement in mitochondrial activity and in acrosomal integrity	Adami *et al* 2018
	5mM	Sample challenged by WIFI radiation	Protection against oxidative stress	Shang-Shu Ding *et al* 2018
Vitamin C	600uM	Samples submitted to oxidative and heat stress	Decrease in oxidative stress status	Ahmad *et al* 2017
Selenium	600uM	Asthenoteratozospermic men	Increase in motility, viability and mitochondrial membrane potential. Decrease in MDA levels and DNA fragmentation	Ghafarizadeh *et al* 2017
Zinc	6mM	Asthenozospermic and Asthenoteratozospermic men	Increase in total antioxidant status	Ajina *et al* 2016
Pentoxifylline	5mM	Normozospermic men	Increased progressive sperm motility and CK activity	Banihani and Abu-Alhayjaa 2015
Myo-inositol	15 ug/mL	Normozospermic and oligoasthenozospermic men	Improvement in sperm motility	Artini *et al* 2016
BDNF	0.133 nM	Normozospermic men	Increase in viability, motility, NO concentration and mitochondrial sperm activity	Safari *et al* 2017
Curcumin	15 uM	Leucocytospermics samples	Improvement in sperm motility	Zhang *et al* 2017
TAT-peroxiredoxin2	100 mg/mL	Asthenozospermic men	Improvement in sperm motility and DNA integrity by reducing levels of ROS	Juan Liu et al 2016
Polyphenols	Hydroxytyrosol 200 ug/mL	Normozospermic men	Improvement in sperm viability and DNA oxidation	Kedechi *et al* 2016
	Propolfenol® 20 and 100 ug/mL	Samples with parameters over the values of 50th percentile, indicated by WHO	Protection against lipid peroxidation	Biagi *et al* 2017
	CAPE 10 -100 uM	Normozospermic and Oligoasthenoteratozospermic men	Reduced MDA levels	Şule Ayla *et al* 2018
	Eruca sativa 15.62 ug/mL	BPA-mediated toxicity	Recovery of the mitochondrial membrane potential	Grami *et al* 2018
	Resveratrol 30uM	Underweight, normal weight, overweight and obese men	Protection against obesity, improving sperm motility	Cui *et al* 2015
	Epigallocatechin-3-gallate 50uM	Testicular tissue	Decrease in oxidative damages (also observed in Sertoli cells)	Dias *et al* 2017

WIFI: Wireless Fidelity; Normozoospermic: normal seminal sample according WHO 2010; H_2O_2: hydrogen peroxide; BPA: Bysphenol-A; Oligoasthenozospermic: low sperm concentration and low sperm motility according WHO 2010; Leucocytospermics: anormal leucocyto amount according WHO 2010; Asthenoteratozospermic: low sperm motility and low sperm normal morphology according WHO 2010; Asthenozospermic: low sperm motility according WHO 2010; CAPE: Caffeic acid phenethyl ester; WHO: World Health Organization

For the rodent section, the search retrieved 152 articles. Appling the exclusion criteria, we selected 52 studies; 3 of them studied varicocele, 11 diabetes, 10 therapeutic drugs, 3 physical exercises, 10 concerned environmental exposure and 3 were about heat stress (Figure 2). The remaining studies (12) involved oxidative stress status in experimental models with rodent animals were used to support the manuscript. All studies of this section can be found in Tables 3, 4 and 5.

Figure 2. Flowchart representing the search and selection of experimental model studies with rodent animal.



Source: Authors.

Table 3. Main factors related to male oxidative stress and substances used to attenuate its effects in experimental models with rodent animals.

Oxidative stress condition	***Substance (dose)***	***Main outcome***	***Reference***
Cigarette smoking	*Achillea millefolium* (120 mg/kg BW/day for 48 days)	Improvement in sperm characteristics (sperm count, motility, dead sperm, and immature sperm), increase of SOD level, decrease of MDA and NO levels, and enhancement of morphometric and morphological parameters.	Salahipour *et al* 2017
Varicocele	Chrysin (50 mg/kg BW/day for 56 days)	Improvement in testicular histology and morphometric parameters. Reduction of sperm DNA fragmentation and testicular MDA levels.	Missassi *et al* 2017
	Alpha-lipoic acid (300 mg/kg BW/day for 60 days)	Restoration of sperm motility and concentration, decrease in DNA fragmentation, and increase in lipid peroxidation.	Shaygannia *et al* 2018
	Royal jelly (200 mg/kg BW/day for 42 days)	Restoration of antioxidant activity (CAT, SOD, GPx), reduction of MDA levels, and decrease of seminiferous tubule injury (Johnsen score improvement).	Asadi *et al* 2019
Cryptorchidism	*Moringa oleífera* (400 or 800 mg/kg BW/day for 14 days)	Decrease or elimination of testicular apoptosis. Reduction of HSP70 expression and MDA levels, and increase SOD levels.	Tekayev *et al* 2018
Obesity	*Caralluma fimbriata* (200 mg/kg BW/day for 90 days)	Preservation of normal testicular histology, prevention of increased levels of LPO and PO, and increase in GSH levels and antioxidant enzymes.	Gujjala *et al* 2016
Diabetes	White tea (administration of the infusion of 1 g/100 mL distilled water in substitution of drinking water for 60 days)	Restoration of testicular protein oxidation and lipid peroxidation, with improvement in antioxidant potential, sperm quality and concentration. Increase of glucose tolerance and insulin sensitivity.	Oliveira *et al* 2015
	Pentoxifylline (12 mg/kg BW/day for 14 days)	Restoration of sperm parameters (sperm count, motility, and abnormality), testosterone and blood glucose levels, and reduction in apoptotic index of testis.	Feyli *et al* 2016
	Ascorbic acid (70 mg/kg BW/day for 30 days)	Reversion of MDA level, prevention of oxidative damage (apoptosis) to sperm and prevention of testicular histological damage. Restoration of germ cell apoptosis. Did not restore testosterone level and motility characteristics.	Aguirre-Arias *et al* 2017
	Lycium barbarum (10, 20 or 40 mg/kg BW/day for 62 days)	Improvement in histological aspects of testis favoring spermatogenesis. Increase in sperm count, sperm viability, and in antioxidants (SOD, GSH-Px, and CAT). Decrease in ROS and MDA levels, Caspase-3 expression, and increase in the Bcl-	Shi *et al* 2017

		2/Bax 43 ratio, which is linked to an anti-apoptotic state.	
	Cerium oxide nanoparticles (30 mg/kg BW/day for 14 days)	Improvement in testicular characteristics, reduction of DNA fragmentation in sperm, and increase in hormonal levels and Nfr2 expression (involved in the regulation of antioxidant defense).	Artimani *et al* 2018
	Loranthus micranthus (100 or 200 mg/kg BW/day for 14 days)	Reduction of the glucose levels. Increase in steroidogenesis. Improvement in sperm quality and antioxidant activity. Restoration of the testis architecture and increase in the expression of BCL-2.	Ebokaiwe 2018
	Crab shell extract (100, 200 or 400 mg/kg BW/day for 14 days)	Increase in the sperm number, motility and testosterone levels. Reduction in NO levels and fasting blood glucose. Increase in FRAP levels.	Ghanbari *et al* 2018
	Lycium barbarum (40 mg/kg BW/day for 62 days)	Improvement in testicular functions under hyperglycemic conditions by regulating the autophagy mechanism in the testes through the PI3K/Akt pathway (downregulation of Beclin-1 and LC31, and upregulation of p-PI3K and p-Akt).	Shi *et al* 2018
	Astragalin (3.3, 10 or 30 mg/kg BW/day for 56 days)	Improvement in testicular architecture and sperm parameters. Reduction of NO and MDA levels. Increase of antioxidant capacity (GSH-Px, SOD, and CAT). Downregulation of TNF-α and iNOS in the testes.	Han *et al* 2019
	Bergenin (from *Ardisia colorata*, at 100, 200, or 400 mg/kg BW/day for 30 days)	Restoration of antioxidant activity and sperm quality. Reduction of DNA fragmentation in sperm.	Sanjeev *et al* 2019
	Tempol (100 mg/kg BW/day for 30 days)	Improvement in histopathological aspects of testis, sperm motility, viability and reduction of abnormality rate. Decrease in fasting blood glucose and LPO. Increase in total antioxidant capacity.	Shateri *et al* 2019
Exercise	E Vitamin (50 mg/kg BW/day for 35 days)	Increase in total testicular antioxidant capacity.	Kalantari *et al* 2017
	Melatonin (10 mg/kg BW weekly for 56 days)	Improvement in progressive motility, reduction in germ cells apoptosis, and increase in antioxidant enzymes.	Moayeri *et al* 2017
	Phyllanthus emblica (50 mg/kg BW once before exercise)	Increase in sperm concentration and testosterone. Decrease in corticosterone levels, MDA, sperm abnormalities, and acrosome-reacted sperm. Enhancement in the testicular steroidogenic acute regulatory (StAR) protein expression.	Arun *et al* 2018
Heat stress	*Mallotus roxbhurghianus* (400 mg/kg BW/day for 14 days)	Suppression of lipid peroxidation. Restoration of antioxidant capacity, testosterone levels, spermatogenesis, and increase in cell proliferation activity.	Roy *et al* 2015
	Fertilix® (dissolved in water, given *ad libitum* for 60 days)	Reduction of sperm DNA damage and reestablishment of pregnancy rates to nearly normal.	Gharagozloo *et al* 2016
	Panax ginseng (100 or 200 mg/kg BW/day for 56 days)	Attenuation of the decreased sperm kinetic values. Improvement in antioxidant-related enzymes, spermatogenesis-related proteins, and sex hormone receptors.	Kim *et al* 2017
	Psidium guajava (100 µl/kg BW/day for 60 days)	Improvement in sperm density, motility and morphology, and on MDA and GSH levels.	Ngoula *et al* 2017

SOD: superoxide dismutase; MDA: malonaldehyde; CAT: catalase; NO: nitrogen oxide; GPx: glutathione peroxidase; HSP70: heat shock protein-70; LPO: lipid peroxidation; PO: protein carbonyl; GSH: glutathione; ROS: reactive oxygen species; FRAP: ferric reducing antioxidant power; BW: body weight; NOS: nitrogen oxide species. Source: Authors.

Table 4. Therapeutic drugs related to semen oxidative stress and substances used to attenuate its effects in experimental models with rodent animals.

Therapy/ Disease	*Oxidative Stress condition*	*Substance (dose)*	*Main Outcome*	*Reference*
Anti-cancer therapy (chemoterapy)	Cyclophosphamide-induced	Peruvian Maca (*Lepidium meyenii*, 500 or 1000 mg/kg BW/day for 28 days)	Reestablishment of testosterone levels, improvement in sperm parameters (sperm count and motility), antioxidant activity, pregnancy rate and litter size	Onaolapo *et al* 2017
		Ghrelin (80 µg/kg BW/day for 35 days)	Improvement in antioxidant status, reduction of MDA levels, and enhancement of sperm parameters.	Salimnejad *et al* 2017
		Acrocomia aculeata (3 or 30 mg/kg BW/day for 35 days)	Mitigation of the toxic effects of cyclophosphamide on testicular tissue, on hormone levels and on the sperm count. Increase of the expression of testicular *Ckit*.	Arena et al., 2018
		Tribulus terrestris (11 mg/kg BW/day for 14 days)	Improvement in biochemical parameters (MDA, SOD, CAT, GPx), sperm parameters (motility, vigor, and integrity), and testosterone levels.	Pavin *et al* 2018
	Cisplatin-induced	Rutin (150 mg/kg BW/day for 14 days, before cisplatin treatment)	Partial increase in sperm motility. Restoration of dead sperm percentage, sperm abnormalities and oxidative/ antioxidant status. Decrease of seminiferous tubule injury (Johnsen score improvement).	Aksu *et al* 2016
	Doxorubicin-induced	Resveratrol (20 mg/kg BW/day for 21 days)	Improvement in sperm parameters (morphology, sperm concentration and motility), and restoration of testosterone and MDA levels.	Türedi *et al* 2015
		Carnitine (250 mg/kg BW in a single dose, before a single dose of doxorubicin)	Protection of acrosome and DNA integrity. Improvement in fertility index and implantation rate.	Cabral *et al* 2017
Immunosuppression	Azathioprine-induced	Taurine-chloramine (100 mg/kg BW/day for 70 days, before azathioprine treatment)	Maintenance of normal levels of hormones (Testosterone, LH, and FSH), and antioxidant status (SOD, CAT, GSH, and MDA). Enhancement of spermatogenesis indices, maintenance of DNA fragmentation, caspase-9 and BCL2 expression similar to control. Partial protection of sperm parameters (motility, vitality, and sperm count).	Shaalan *et al* 2018
Epilepsy	Valproic acid-induced	Resveratrol (10 mg/kg BW/day for 28 days)	Prevention of impairment in sperm motility. Decrease in oxidative damage. Increase in the antioxidant status of testes and epididymis.	Ourique *et al* 2016
Antiprotozoal/ Antibacterial therapy	Metronidazole-induced	Rosmarinic acid (5 mg/kg BW/day for 30 days)	Improvement of testicular structure and sperm parameters (morphology, sperm count, and motility).	Al-Alami *et al* 2017

SOD: superoxide dismutase; MDA: malonaldehyde; CAT: catalase; LH: Luteinizing hormone; GPx: glutathione peroxidase; FSH: Follicle stimulating hormone; BW: body weight. Source: Authors.

Table 5. Environmental agents related to semen oxidative stress and substances used to attenuate its effects in experimental models with rodent animals.

Environmental exposure agent	*Substance (dose)*	*Main Outcome*	*Reference*
Arsenic	Vitamin C (100 mg/kg BW/day) or zinc chloride (20 mg/kg BW/day) for 60 days	Protection of morphometric and morphologic aspects of testes. Improvement in sperm production. Protection of sperm against morphological defects.	Altoé *et al* 2016
	α-lipoic acid (70 mg/kg BW, 3 times a week for 8 weeks)	Improvement of total sperm count, sperm viability and motility, in addition to testosterone concentration.	Prathima *et al* 2017
	Chasmanthera dependens (25, 50 or 100 mg/kg BW/day for 60 days)	Increase in sperm parameters (semen volume, sperm count, viability, and motility). Reduction of sperm defects. Increase in testosterone and antioxidant levels. Enhancement of fertility aspects.	Quadri and Yakubu 2017
Lead	Punicalagin (9 mg/kg BW/day for 28 days)	Enhancement of sperm characteristics, antioxidant status and upregulation of Nrf2 expression.	Rao, Zhai and Sun 2016
Aflatoxin	Selenium (0.4 mg/kg BW/day for 45 days)	Partial reversion of aflatoxin effects on sperm parameters (motility, concentration, and morphology), levels of testosterone, ROS, MDA, and GSH activity. Increase in expression of steroidogenic enzymes StAR, P450scc and 17β-HSD.	Cao *et al* 2017
Fungicide	*Ionidium suffruticosum* (250 mg/kg BW/day for 28 days)	Reestablishment in sperm vitality and morphology, and in levels of antioxidant enzymes (CAT and SOD), with a decrease of MDA level.	Chenniappan and Murugan 2017
BPA	E Vitamin (40 mg/kg BW/day for 90 days)	Improvement in weight and histology of testis, testosterone levels, sperm count and apoptosis in epididymal sperm.	Srivastava and Gupta 2018
	Selenium (3 mg/kg BW/day) or nano-selenium (2 mg/kg BW/day) for 70 days	Attenuation of the reproductive toxicity induced by BPA via improvement of antioxidant activity, genetic changes, and restoration of testis tissue (similar to control).	Khalaf *et al* 2019
PVC	Resveratrol (20 mg/kg BW/day for 60 days)	Improvement in steroidogenesis and spermatogenesis. Mitigation of oxidative stress (lipid peroxidation, SOD, and CAT). Enhancement in sperm parameters (sperm count, motility, and viability) and fertility characteristics (conception time and number of implantations/rat).	Archana *et al* 2018
γ-rays	Silymarin (50 mg/kg BW/day for 7 days before radiation)	Protection from testes weight loss, damage of sperm chromatin and reduction on sperm count. Normal morphology and progressive motility.	Fatehi *et al* 2018
Pollution (particulate matter 2.5)	Vitamin C (100 mg/kg BW/day) and E Vitamin (50 mg/kg BW/day) for 28 days	Increase in conception rate and sperm concentration. Decrease in the abnormality rate. Improvement in biochemical analysis (MDA and SOD). Decrease in the toxicity of pollution by presenting a more adequate pattern of proteins expression.	Liu *et al* 2019

SOD: superoxide dismutase; MDA: malonaldehyde; CAT: catalase; GSH: gluthationa; ROS: reactive oxygen species; BPA: bisphenol-A; BW: body weight; PVC: Polyvinyl chloride. Source: Authors.

4. Discussion

4.1 Redox balance in human sperm

Under physiological condition, ROS are essential to several fertilization processes, such as hyperactivation, sperm capacitation, sperm-oocyte interaction, fertilization *per se*, cellular activation, and maintenance of cellular fluidity and homeostasis (Banihani et al., 2018; Moretti et al., 2017). However, when an oxidative imbalance occurs, either due to the increase in total pro-oxidative agents or by the decrease of the cellular antioxidant capacity, it starts a process called oxidative stress (OS) (Gulum et al., 2017; Riaz et al., 2016). Spermatozoa is highly susceptible to OS since it has extremely reduced

cytoplasmic volume, resulting in low antioxidant capacity. In addition, sperm's plasma membrane is rich in polyunsaturated fatty acids which makes it more susceptible by ROS action, and this action can be toxic to sperm (Sposito et al., 2017).

Studies have shown that OS, directly or indirectly, involves in 50% of male infertility cases. The oxidative imbalance found in various conditions like idiopathic infertility (Mayorga-Torres et al., 2017), varicocele, genitourinary infections (Micheli et al., 2016), testicular tumor (Sposito et al., 2017) and lifestyle habits like smoking (Bassey et al., 2018; Ranganathan et al., 2018) or environmental exposures (Kasperczyk et al., 2016a) can impair cellular functions, resulting in problems in spermatozoa and its microenvironment, further leading to infertility (Kolesnikova et al., 2015, 2017). Moreover, OS can promote imbalance in inflammatory factors such as pro-inflammatory cytokines (Chyra-Jach et al., 2018; Dobrakowski et al., 2017).

Many couples who seek ART have higher rates of DNA fragmentation, with impairment in DNA repair enzymes (Atig et al., 2017; Dorostghoal et al., 2017; Kamkar et al., 2018; Wdowiak et al., 2015), as well as an increase in lipid peroxidation (Ahelik et al., 2015; Sposito et al., 2017). In those cases, the preferred approach is: test application to identify the infertility causes, then antioxidant supplementation to control oxy-redox potential in both seminal donation rates (Ahelik et al., 2015; Sposito et al., 2017) and cryopreservation mediums used in ART (Panner Selvam et al., 2018).

In order to better understand the best threshold to separate fertile and infertile men, several different approaches were tested. A study analyzed sperm function of both groups (Mayorga-Torres et al., 2017), other evaluated the antioxidant enzymatic profile in comparison to normal sperm parameters (Macanovic et al., 2015; Pajovic et al., 2016; Silberstein et al., 2016), and other tried to establish the ideal amounts of ROS in the semen (Agarwal et al., 2015). Using techniques like chemiluminescence, Agarwal and colleagues analyzed the semen of 258 infertile men and established the cutoff of 102.2 RLU/s/106 (relative light units), with sensitivity of 76.4% and specificity of 53.3% (Agarwal et al., 2015)**.** Using colorimetric methods, Roychoudhury and colleagues established the cutoff of 1947 μM (Trolox equivalents) of total antioxidant capacity in seminal plasma, with 63% specificity and 59.5% sensitivity (Roychoudhury et al., 2016).

Knowing the normal concentration of certain antioxidants and their main enzymes, as well as the total antioxidant capacity of sperm cells, allows performing correlations with oxidative status, and promoting antioxidant response to oxidative stress (Atig et al., 2017; Bousnane et al., 2017; Moretti et al., 2017). One example that was adopted by Dorostghoal and colleagues is a cutoff of MDA 4.2 nmol/ml, SOD 4.89 U/ml and GPx 329.6 mU/ml (Dorostghoal et al., 2017). Kratz and colleagues, in 2016, used radioimmunoassay and spectrophotometric methods to associate the presence of melatonin, an antioxidant, to sample oxidative status (Kratz et al., 2016), as well as its seasonal alterations that did not interfere in sperm DNA fragmentation index (Malm et al., 2017)**.** Evidokimov and colleagues found that 10uM of hydrogen peroxide (H_2O_2) semen addition could provide optimal functioning of pentose pathway enzymes and their antioxidant defenses (Evdokimov et al., 2015)**.** In addition, the remarkable presence of some ions that are also cofactors of enzymatic reactions, such as iron, zinc, copper, and selenium, seem to impact semen oxidative status and to be more evident in non-normal samples (Dobrakowski et al., 2017; Kasperczyk et al., 2016b; Nenkova et al., 2017).

4.1.1 Premises for antioxidant therapy

In spite of several researches investigating antioxidant therapies as alternatives to prevent oxidative stress, studies using this kind of treatment in sperm samples have shown controversial results; some observing improvement, others showing inconclusive results, and some obtaining negative results (Alsalman et al., 2018; ElSheikh et al., 2015; Rago et al., 2017; Salian et al., 2019; Skibińska et al., 2016; Yamamoto et al., 2017). To achieve a successful antioxidant treatment, several factors should be accounted. The first is whether the concentration of oxygen species overcomes the antioxidant defense systems, meaning that the cellular system must be in OS (Atig et al., 2017; Bousnane et al., 2017; Mayorga-Torres et al., 2017;

Moretti et al., 2017). In addition, it is crucial to consider that a specific antioxidant system will neutralize each ROS and, since the susceptibility of sperm to ROS can vary between species, it is vital to acknowledge which ROS is more harmful to the given specie in order to conduct a targeted antioxidant therapy (Agarwal et al., 2015; Roychoudhury et al., 2016). Therefore, an unspecific antioxidant treatment may not be efficient to the ROS in one sample or even have a toxic effect. Moreover, different sperm structures may be more or less susceptible to the action of specific ROS, suggesting that, it is necessary to associate antioxidants to get a synergistic action and prevent oxidative damage to all structures (Giacone et al., 2017). Finally, it is crucial to determine the ideal concentration of each antioxidant before using in therapy since low concentrations may not be sufficient to maintain cellular oxidative homeostasis, and high concentrations can inhibit the physiological ROS, promoting a deleterious effect on sperm and fertilization processes (Dias et al., 2015). Therefore, for this treatment to be efficient, it is fundamental to perform an association of antioxidants with specific concentrations required for each ROS, increasing its complexity and possibly explaining the variability in the results of published studies.

4.1.2 Oral antioxidant supplementation

Oral antioxidant supplementation is a well-regarded practice that has been used by medical recommendations, including for treatment of infertile couples and post-surgical treatments (Cyrus et al., 2015; Eslamian et al., 2017). The Vitamin C, (i.e. ascorbic acid) that can be found in fruits and vegetables, is a powerful antioxidant neutralizing hydroxyl radical. However, it loses its biological properties quickly at inadequate temperatures (Bouzari et al., 2015; Phillips et al., 2018). This vitamin also contributes to hydroxylation of collagen and immune defense in the body, being one of the most used vitamins for oral supplementation (Carr & Maggini, 2017; Padayatty & Levine, 2016). Cyrus and colleagues proposed in 2015 a protocol of vitamin C supplementation to men from 18 to 50 years old, operated on varicocele by inguinal method (Ivanissevich). After the surgery, the study group (46 men) received 250 mg of vitamin C while the control group received placebo; the supplementation lasted 3 months. Seminal parameters were evaluated according to WHO 1999, with 3 days of ejaculatory abstinence. Vitamin C supplementation increased sperm motility and normal cells, even after controlling for confounders, suggesting an adjuvant potential in treatment after varicocelectomy (Cyrus et al., 2015). In addition, the same treatment showed an inverse correlation between levels of vitamin C and myeloperoxidase concentration in seminal plasma, being this protein a producer of oxidants (Pullar et al., 2017). In the same way, Rafiee and colleagues observed that 1g of vitamin C daily administration for 6 months, improved sperm concentration and motility in men between 20 and 60 years old (Rafiee et al., 2016). Gual-Frau and colleagues (2016) performed a 3-month daily multivitamin (1500 mg L-Carnitine, 60 mg of vitamin C, 20 mg of Q10 coenzyme, 10 mg of vitamin E, 200 μg of vitamin B9, 1 μg of vitamin B12, 10 mg of zinc, 50 μg of selenium) supplementation in 20 men with grade I varicocele. Despite no differences in seminal parameters (WHO 1999), the DNA fragmentation of control group, assessed using the Sperm Chromatin Dispersion (SCD) test (Halosperm Kit, Halotech DNA, S.L; Madrid, Spain), was 22.1% lower than in the multivitamins group. Besides, 2 of the 20 infertile couples in this study got pregnant after treatment, suggesting the multivitamin might have worked as a DNA fragmentation restorer (Gual-Frau et al., 2015).

Vitamin E (alpha-tocopherol) is a potent liposoluble, non-enzymatic antioxidant (Adami et al., 2018), which is present in the spermatic environment and assists in ROS neutralization (Gvozdjáková et al., 2015)**.** Alpha-tocopherol reacts with free radicals, producing alpha-tocopheroxyl, which neutralizes free radicals by reacting with other molecules of alpha-tocopheroxyl. Because of that property, Ener and colleagues treated 22 inguinal varicocelectomized men with 600mg of vitamin E for 1 year and found improvement in seminal parameters, but did not find differences comparing to the control group (that did not receive this treatment) (Ener et al., 2016). Daily vitamin E addition (400mg) to antiestrogen therapy with clomiphene citrate (25 mg) during 6 months, improved sperm concentration and motility in oligoasthenozospermic men

(ElSheikh et al., 2015). Moreover, adding healthy habits like the practice of physical activities to cosupplementation treatment (400mg Vit E + 1g Vit C + 2g L-carnitine) showed promising results concerning seminal parameters (Magdi et al., 2017).

Many authors advocate for a balanced diet with supplementation of nutrients; they also believe that the best approach is combining two or more substances, decreasing male infertility risk, as occurs in asthenozoospermia cases (Eslamian et al., 2017)**.** Gvozdjáková and colleagues, in 2015, treated 40 men with Carni-Q-Nol (440mg L-carnitine fumarate + 30mg ubiquinol + 75 IU vitamin E + 12mg vitamin C in each soft sole) and reported there was greater sperm density on seminal samples as well as the pregnancy rate after 6 months of treatment was 45% (Gvozdjáková et al., 2015). In the same way, associating 440 mg of l-carnitine with 250 mg of l-arginine, 40 mg of zinc, 120 mg of vitamin E, 80 mg of glutathione, 60 μg of selenium, 15 mg of Q10 coenzyme and 800 μg of folic acid in a once-a-day treatment during 3 months seems to elevate sperm density and the motility of spermatozoa (Lipovac et al., 2016). The same happened when men with varicocele took 1g of L-carnitine + 725 mg of fumarate + 500 mg of acetyl-L-carnitine + 1,000 mg of fructose + 20 mg of CoQ10 + 90 mg of vitamin C + 10 mg of zinc + 200 μg of folic acid + 1.5 μg of vitamin B12 and showed an improvement in sperm concentration and motility (Busetto et al., 2018). Moreover, another study found erectile function improvement in Japanese men after 4 months of treatment with a combination of L-arginine (690 mg) and French maritime pine bark extract (Pycnogenol® 60mg) involved in NO way (Kobori et al., 2015). Although one study has not found improvement in oocyte numbers, fertilization rate and embryonic quality in couples undergoing Intracytoplasmatic Sperm Injection (ICSI) after association of other micronutrients (a.g, alpha lipoic acid and glutathione), this therapy lowered the risk of miscarriage, which suggests that supplements may improve/support assisted reproduction techniques (Rago et al., 2017)**.**

Antibiotics (Ahmadi et al., 2017) and other nutrients with antioxidant function used in oral treatments may impact some parameters of asthenozoospermic men. The examples and results found in this review were: alpha-lipoic acid (600mg for 3 months) improved total count, concentration and sperm motility, as well as total antioxidant capacity (Haghighian et al., 2015)**;** tamoxifen (10mg) improved mitochondrial membrane potential and antioxidants enzymes (Guo et al., 2015); Zinc (440mg daily) normalized the activity of thiol-related enzymes in infertile patients (Alsalman et al., 2018); lycopene (30mg) decreased white blood cells in seminal plasma and increased lycopene plasmatic concentration (Yamamoto et al., 2017)**;** N-acetyl-cysteine (600mg daily) decreased abnormal cells count, DNA fragmentation and protamine deficiency (Jannatifar et al., 2019); docosahexaenoic acid (1.5g per day during 10 weeks) reduced DNA damage (Martínez-Soto et al., 2016); curcumin (80mg daily) improved total antioxidant capacity, malondialdehyde, C-reactive protein and tumor necrosis factor (Alizadeh et al., 2018), and finally, antioxidant probiotics (Valcarce et al., 2017) decreased H_2O_2 intracellular levels.

4.1.3 *In vitro* antioxidant supplementation

Many fundamental researches adopt *in vitro* antioxidant supplementation to better understand its functioning and possible influence on sperm quality (Ahmadi et al., 2017; Bousnane et al., 2017). This happens when some products such as antioxidants are added in the human seminal sample after ejaculation. This event may be during the processes involved in assisted reproduction techniques, such as cryopreservation and/or seminal processing for semen preparation and for choosing the best gamete to fertilization. Hydro soluble vitamin B (complex) acts on carbohydrates metabolism as a cofactor for CO_2 transport, playing an essential role in growth, development and DNA, RNA protein, and phospholipids repair (Mikkelsen & Apostolopoulos, 2018). With that in mind, Salian and colleagues collected seminal samples from 238 normo and asthenozoospermic men from 24 to 47 years old and used the swim-up technique to select the mobile spermatozoa. Samples were incubated with 10nM of biotin for 1 hour, which leads to better sperm motility and allowed the proposition of a new experimental model with better fertilization rates and embryonic quality (Salian et al., 2019).

A commonly used approach is testing different treatments in stress-induced samples. For instance, Adami and colleagues challenged seminal samples from 30 normozoospermic men with an oxidative stress inducer (5mM of H_2O_2), increasing DNA fragmentation and superoxide anion (O_2^-) production. However, supplemented samples with 40uM of vitamin E had improvement in mitochondrial activity (percentage of mitochondria active through cytochrome c oxidase activity) and acrosomal integrity (Adami et al., 2018). Similar results were found by Shang-Shu Ding and colleagues, in which 20 seminal samples were exposed to 2.45 GHz Wi-Fi frequency, inducing functional impairment that was reversed with the supplementation of 5mM of vitamin E (Ding et al., 2018). Vitamin C is also widely used for seminal supplementation to inhibit the DNAse-I, one of the major endonucleases involved in DNA fragmentation (Ilić et al., 2018). Ahmad and colleagues in 2017 decreased the oxidative status of human seminal samples submitted to heat stress (34.5°, 37° and 39.5°C) and oxidative stress (200uM of H_2O_2) using vitamin C supplementation (600uM) (Ahmadi et al., 2017).

Selenium and zinc are other commonly used antioxidants as an *in vitro* supplements. The bioavailability of trace elements plays a beneficial role in antioxidant response since they influence the activity of corresponding enzymes. Some examples are the enzyme superoxide dismutase (SOD) and its cofactors copper and zinc, and the enzyme glutathione peroxidase (GPx) with its cofactor selenium (Nenkova et al., 2017). Selenium is incorporated as a prosthetic group in catalytic sites forming selenoproteins, such as the enzyme GPx (Nenkova et al., 2017). GPx4 plays a critical role in male fertility, contributing to the architecture of the midpiece of sperm and protecting sperm from ROS action during spermatogenesis. Moreover, GPx can reduce oxidation by reacting with oxidizing factors (Ghafarizadeh et al., 2018). The relationship of zinc-SOD is important for enzymatic anti-oxidative defense, since SOD catalyzes the conversion of superoxide radicals into H_2O_2, preventing the lipid peroxidation of the plasma membrane (Nenkova et al., 2017)**.**

Ghafarizadeh and colleagues incubated samples of 50 asthenozoospermic men with 2ug/mL of selenium at 37°C for 2 and 4 hours and reported improvement in sperm motility, vitality, membrane potential, as well as a decrease in sperm DNA fragmentation when samples were incubated for 6 hours (Ghafarizadeh et al., 2018). Furthermore, Ajina and colleagues showed an increase in the total antioxidant capacity of 38 samples of infertile men incubated with 6mM of zinc for 2 hours at 37°C (Ajina et al., 2017). Pentoxifylline, which is a xanthine derivative, has been adopted in clinics as a method of selecting viable spermatozoa in assisted reproduction treatments (ART) (Banihani et al., 2018), and associated with the enzyme creatine kinase to improve motility (Banihani & Abu-Alhayjaa, 2016). Despite some concerns regarding its use (Unsal et al., 2016), in one study that followed 102 patients on ICSI cycles, using pentoxifylline did not impact the rates of malformation in live births (Navas et al., 2017).

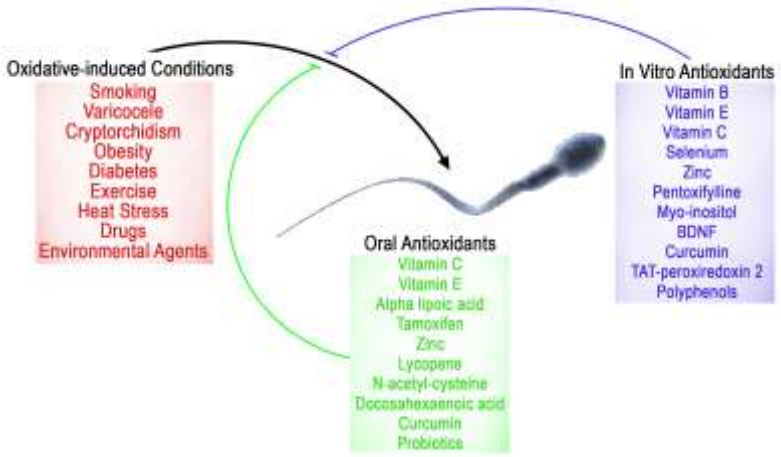
Other substances have been used more recently as *in vitro* supplementation of human seminal samples. The Myo-inositol (15ug/mL) improved motility in 73 seminal samples from normo and oligoasthenozoospermic men between 25 and 45 years old (Artini et al., 2017). Some peroxiredoxins act like ROS scavengers (H. Shi et al., 2018), being required to promote better spermatozoa capacitation (Lee et al., 2017). One example is the fusion protein TAT-peroxiredoxin 2, which improves sperm motility and DNA fragmentation (J. Liu et al., 2016). Moreover, one study showed that 0.133nM of brain-derived neurotrophic factor (BDNF) supplementation in 20 normal seminal samples increased sperm motility and vitality, and decreased the NO and malondialdehyde (MDA) levels (Safari et al., 2018). Incubation of 15uM of curcumin during 2 hours lead to motility improvement in leucocytospermic samples (L. Zhang et al., 2017). Other remarkable examples are: polyphenols like the hydroxytyrosol (200ug/mL) present in olive oil significantly improved sperm viability and DNA oxidation (Kedechi et al., 2017); Propolfenol® (20 and 100ug/mL) protected against sperm lipid peroxidation (Biagi et al., 2018); caffeic acid phenethyl ester (CAPE, present in propolis) (10-100uM) reduced MDA levels (Ayla et al., 2018); extract of the herb *Eruca Sativa* remarkably improved the mitochondrial membrane potential in samples treated with Bisphenol A (Grami et al., 2018); resveratrol (30uM) protected from obesity-induced OS condition and improved sperm motility (Cui et al.,

2016); and epigallocatechin-3-gallate (50uM) present in green tea decreased oxidative damages in Sertoli cells (Dias et al., 2017).

Some authors recommend the combination of 2 or more substances with antioxidant function to treat *in vitro* samples. Giacone and colleagues proposed in 2016 the application of 0, 500, and 40 µg/ml of Zn+ D-Asp and Co-Q10 combined, reporting that the incubation of 24 seminal samples for 3 hours decreased lipid peroxidation and increased sperm motility (Giacone et al., 2017). This improvement can be explained by the regulation of type Hv1 way proton channels, discovered by Chae and colleagues when they analyzed incubation with 50 lg/mL of quercitin, *Allium cepa L.* peel extract, during 3 hours (Chae et al., 2017). Moretti and colleagues used a similar protocol and found acrosome and membrane preservation (Moretti et al., 2016).

On the other hand, there is a considerable discussion concerning the real benefits of using antioxidants, oral or *in vitro*, as protective factors against oxidative stress (Skibińska et al., 2016). Thereby this controversy highlights the importance of knowing the best substances, their ideal concentrations, and the ideal incubation times (figure 3). Dias and colleagues disclosed that the incubation concentration of caffeine supplementation influences seminal samples in a dose-dependent manner (Dias et al., 2015). Another study showed that utilizing rosmarinic acid, a phenolic ester jeopardizes sperm function depending on the dose (Lv et al., 2018).

Figure 2. Figure representing some kind of oral and in vitro antioxidant, and the oxidative-induced conditions.



Source: Authors.

4.2 Oxidative stress and experimental models

This section will broadly discuss experimental animal model key role *i.e.* mainly the use of rodents to study oxidative stress. Animal models are used in male fertility studies to understand how different conditions (*e.g.* diabetes, obesity, and others) can reduce the fertile potential, and the role of different drugs and substances in reverting these conditions. This section is divided according to the main factors that oxidative stress impact on testicular, however, even though some factors like cigarette smoking are known to impair male reproductive potential (Antoniassi et al., 2016), few studies assess their relationship with oxidative stress in experimental models. For this reason, cigarette smoking was kept out from the subsections.

Cigarette smoking may induce male reproductive disorders, mainly by nicotine exposure. In male adult rats, nicotine exposure (0.2 and 0.4 mg/kg/day) reduces antioxidant capacity and SOD levels, besides increasing nitrite and MDA levels (Salahipour et al., 2017). Sperm parameters like sperm count and motility are also affected, followed by an increase in the levels of dead and abnormal sperm. This condition may be partially reverted using *Achillea millefolium*, which also increases SOD production (Salahipour et al., 2017).

Obesity is another condition that negatively affects testicular function. Gujjala and colleagues (2016) observed a protective effect of *Caralluma fimbriata* and metformin on the oxidative stress caused by high-fat diet, showing a decrease in lipid peroxidation (LPO) and protein oxidation. Furthermore, the authors reported that the administration of *C. fimbriata* with a high-fat diet partially prevented the loss of weight of testis and epididymis, which could impair spermatogenesis. At the testis level, they demonstrated by histopathological analysis that high-fat diet-induced seminiferous tubules atrophy in rats, with a sperm absence condition in the lumen of those tubules (Gujjala et al., 2016). In rats with induced hepatic steatosis, the use of *Crataegus aronia* preserved testicular architecture, improving sperm parameters (Dallak, 2018).

Evidence suggests that other conditions or pathologies related to oxidative stress may benefit from antioxidant therapy. Rats with surgically induced unilateral cryptorchidism showed high levels of MDA, apoptosis, and heat-shock proteins (HSP70), as well as decreased levels of SOD (Tekayev et al., 2019). Using the extract of *Moringa oleifera*, a powerful natural antioxidant rich in β-carotene, riboflavin, folic and nicotinic acids and vitamins A, B C and E, the authors found an important reduction in oxidative stress, in the expression of HSP70 (a heat shock protein which is involved in sperm cell compromise) and apoptosis of germ cells (Tekayev et al., 2019). A study sought to evaluate the role of 3 compounds (dihydroquercetin, p-tyrosol, dibornol) in rat infertility induced by pathospermia (Borovskaya et al., 2018). Among them, the most effective was dibornol, which significantly improved sperm count, motility, and antioxidant capacity.

The body has its own defenses against testicular oxidative stress. A recent study discovered the expression of both mRNA and protein Nesfatin-1 in the testis of adult mice, being the expression of this hypothalamic neuropeptide correlated to steroidogenic markers (Ranjan et al., 2019). In this study, *in vitro* treatment of testis with Nesfatin-1 stimulated spermatogenesis and reduced oxidative stress and NO levels, improving testicular function.

The use of antioxidants to reduce testicular oxidative stress and, consequently, mitigate its negative impact on semen, can increase natural conception and ART. Although most of the studies we will discuss below have used *in vivo* therapies to treat experimental models, there might be a remarkable benefit of *in vitro* antioxidants. For instance, in mice, supplementation of sperm washing medium with biotin or pentoxifylline improved the rate of fertilization and blastocysts (Salian et al., 2019).

From the data exposed in this review, we identified noticeable participation of oils and plant extracts in antioxidant therapies. In addition, these herbs usage as antioxidants has a regional characteristic since some of them are native to specific countries or regions. Several cultures locally recognize native herbs as “health enhancers” or libido and fertility boosters. One study sought to test the hypothesis that one of these herbs, the leaf of a Nigerian walnut (*Tetracarpidium conophorum*) could improve the fertility of rats. In this case, an improvement was demonstrated in all characteristics linked to fertility: seminal, hormonal, DNA fragmentation, and antioxidant capacity (Akomolafe & Oboh, 2017). Similar results were also observed when caffeine and caffeic acid were co-administered (Akomolafe et al., 2018).

Next, we address some conditions that are known to induce oxidative stress and list the antioxidants tested aiming improving these conditions.

4.2.1 Varicocele

Varicocele is a condition commonly associated with male infertility, being present in approximately 35% with primary and up to 80% with secondary infertile men (when the men have children previously). It is characterized by abnormal dilatations in the pampiniform plexus veins, leading to testicular temperature increase. Hence, its association with oxidative stress results from enhanced ROS and LPO products, such as MDA (Asadi et al., 2019). Although this condition can be repaired by a surgical procedure known as varicocelectomy, restoring seminal and hormonal profiles *albito* in most of the cases, its effects on oxidative stress are still under debate. For this reason, antioxidant adjuvant therapy still has been considered a relevant strategy and an open research area.

Two studies highlighted the use of honey derivatives as antioxidants (Asadi et al., 2019; Missassi et al., 2017). Crysin, a flavonoid constituent of honey, was found to enhance sperm parameters, such as DNA fragmentation, and protect varicocele-induced rats from OS (Missassi et al., 2017). In this study, authors found that when Crysin was administrated to varicocele-induced rats, histological characteristics of the testis were improved, characterized by the maintenance of normal seminiferous tubule with tubules diameter compared to control. Moreover, Crysin decreased sperm DNA fragmentation induced by varicocele, and OS assessed by MDA concentration (Missassi et al., 2017). Later, Asadi and colleagues (2019) evaluated MDA, SOD, catalase (CAT), and GPx levels in varicocele-induced rats treated with 200 mg/kg of royal jelly. Animals that received royal jelly after surgery had higher antioxidant activity (*i.e.* CAT, SOD, and GPx), total antioxidant capacity, and a significant reduction in MDA levels. Thus, besides the deleterious effects of varicocele induction on sperm parameters, royal jelly improved sperm viability. Moreover, using Johnsen's score to evaluate spermatogenesis in a histological assessment, authors disclosed that royal jelly kept the spermatogenesis levels close to the physiological range (Asadi et al., 2019).

Other compounds were candidates to improve oxidative conditions. Alpha-lipoic acid (ALA), a disulphide extently studied in other pathologies (*e.g.* diabetes, Alzheimer), reverted the hazardous effects of varicocele induction in rats (Shaygannia et al., 2018). In this study, treatment with 300 mg/kg of ALA 2 months after surgery preserved testicular volume loss and epididymal length, thus improving sperm concentration and motility compared to non-treated varicocele-induced rats. Other parameters such as DNA fragmentation percentage, sperm chromatin condensation and maturation were also improved (Shaygannia et al., 2018).

4.2.2 Diabetes

Diabetes mellitus (DM) is a chronic metabolic disease that affects multiple systems, including reproductive system. It can be caused by a deficiency in insulin production (type 1 DM) or action (type 2 DM). The reproductive disorders associated with DM include sexual dysfunction and infertility, which in turn could be related to oxidative damage.

As Han and colleagues (2019) described, hyperglycemia consequent from DM leads to an excessive generation of ROS, which in turn impairs antioxidant system and disrupts testicular function. Moreover, it may induce a pro-inflammatory status with the participation of tumor necrosis factor-α (TNF-α). Among the flavonoids from *Cuscuta Chinensis*, astragalin was found to enhance in testicular architecture, antioxidant capacity and downregulate TNF-α expression and inducible NO synthase enzyme in the testis (Han et al., 2019).

Several studies revealed important findings in improving OS caused by diabetes. For instance, Feyli and colleagues (2016) demonstrated that pentoxifylline administration, a methylxanthine involved in the anti-inflammatory response, could restore sperm count, motility and abnormalities, testosterone and glucose serum levels, and reduce apoptotic index in testis (Feyli et al., 2017). On the other hand, ascorbic acid seems to have less remarkable effects, since no impact was found regarding testosterone level restoration and sperm motility improvement (Aguirre-Arias et al., 2017).

Bioproducts, mainly derived from animals or plants, are commonly used due to their antioxidant activity. For instance, *Lycium barbarum* (goji berry) has been tested as a possible intervention in two recent publications concerning male reproduction. The authors highlighted goji berry key role in reducing testicular OS caused by diabetes (G.-J. Shi et al., 2017; H. Shi et al., 2018). In the first article, oral administration of *L. barbarum* for 62 days to male mice had a protective effect on spermatogenesis, with improvement of testicular histological aspects. Other benefits were also observed, i.e. an increase in sperm count and viability, a decrease in ROS and MDA levels, and a marked increase in the antioxidants SOD, GSH-Px and CAT (G.-J. Shi et al., 2017). In the second article, the authors found a decrease in Caspase-3 expression and an increase in the Bcl-2/Bax ratio, which is linked to an anti-apoptotic state. *L. barbarum* also improved testicular functions under hyperglycemic conditions, by regulating the mechanism of autophagy in testes through PI3K/Akt pathway (H. Shi et al., 2018).

Other plant extracts are also involved in reducing the OS induced by DM. *Loranthus micranthus* is a Nigerian herb commonly used in African traditional medicine. Using diabetic male Wistar rats, Ebokaiwe and colleagues (2018) showed that treatment with *L. micranthus* reduced glucose levels considerably, followed by an increase in steroidogenesis, improvement in sperm quality and antioxidant activity. This treatment also restored testes architecture and increased BCL-2 expression, being this protein responsible for preventing apoptosis by blocking the mitochondrial cytochrome C release (Ebokaiwe et al., 2018). Marlberry (*Ardisia colorata*) is a herb from the Asian Southeast. It is locally used to treat DM. The antioxidant activity of bergenin, a phytochemical constituent of marlberry, was also tested in Wistar rats (Sanjeev et al., 2019), showing its potential to restore the sperm quality impaired, antioxidant activity, and reduce sperm DNA fragmentation.

The consumption of white tea was also tested in prediabetic rats, resulting in restoration of testicular protein oxidation and LPO, improvement of the antioxidant potential and sperm quality and concentration. It had a considerable impact on glucose tolerance and insulin sensitivity (Oliveira et al., 2015). Although this and other studies have highlighted the efficiency of natural extracts – which could be a low-cost and interesting approach to treat male infertility – further studies are still needed until it becomes a clinical recommendation.

From the diversity of nutritional supplements with promising results exposed here, it seems clear that they can play a role in improvement of infertility caused by diabetes. It was further demonstrated that crab shell extract could increase sperm number and motility, testosterone levels, reduce NO levels, and fasten blood glucose (Ghanbari et al., 2019).

Finally, synthetic antioxidants may also influence diabetes parameters; one of them is tempol (Shateri et al., 2019). It reduces fasting blood sugar and enhances the antioxidant capacity, which favors better sperm characteristics like motility, viability, and lower level of sperm abnormalities.

4.2.3 Therapeutic drugs

Different drugs can induce OS, among which the ones used in anti-cancer therapy are considered the strongest. This happens because chemotherapy targets cells that are in constant proliferation. Consequently, germ cells, that are constantly dividing, end up being targeted as a side effect. Various chemotherapeutic drugs are commonly used to treat cancer, including cisplatin, cyclophosphamide, and doxorubicin. Three different articles recently published in a same journal (Arena et al., 2018; Onaolapo et al., 2018; Salimnejad et al., 2018) demonstrating similar effects in enhanced OS condition caused by cyclophosphamide administration with different agents co-administration. For example, in mice it has also been suggested that the previous use of *Tribulus terrestris*, a natural and potent testosterone stimulant, may protect the male reproductive system from cyclophosphamide-induced damage (Pavin et al., 2018).

Salimnejad and colleagues (2017) showed that ghrelin, a peptide hormone produced in the stomach, positively affects mice treated with cyclophosphamide. The authors improved antioxidant capacity, reduced MDA levels, and enhanced sperm parameters, thus potentiating the fertilizing capacity. Subsequently, the use of Peruvian Maca (*Lepidium meyenii*), a tuber with aphrodisiac properties and known for being beneficial for fertility, was tested in mice to mitigate the toxic effects of cyclophosphamide (Onaolapo et al., 2018). Maca supplementation reestablished testosterone levels and increased sperm count and motility, antioxidant activity, and pregnancy rate. In both studies, even in the groups that were not treated with cyclophosphamide, the supplement improved some sperm parameters and fertility. Finally, in the third study, rats exposed to cyclophosphamide were treated with the oil extracted from a Brazilian plant *Acrocomia aculeata*, that has powerful antioxidants in its composition, such as β-carotene and α-tocopherol (Arena et al., 2018). The authors reported that the oil use mitigated the toxic effects of cyclophosphamide on testicular tissue, hormone levels, and sperm count. Furthermore, it increased the expression of testicular *Ckit*, an important gene for spermatogenesis for being involved in cellular proliferation and differentiation.

The use of antioxidants seems to be likewise promising to reduce the OS caused by less commonly studied chemotherapy drugs. For instance, the damage caused by treatment with cisplatin was reduced by Rutin flavonoite (Aksu et al., 2017), and the harmful effects of azathioprine were nearly eliminated by pretreatment with taurine (Schaalan et al., 2018). Other chemotherapeutic agents, like doxorubicin, induced testicular OS. Rats treated with this drug had a significant impairment in sperm concentration, motility, and morphology, as well as a reduction in plasma testosterone and increased levels of MDA. However, in groups that co-administered resveratrol, a natural antioxidant found in various plants (e.g. grape and peanut), these parameters were similar to the untreated control (Türedi et al., 2015). A protective effect against doxorubicin-induced OS was also observed with the use of carnitine (Cabral et al., 2018). In this study, the authors disclosed that when used before chemotherapy, carnitine had a protective effect on acrosome and DNA integrity, as well as an improving effect on the fertility index and implantation rate.

Epilepsy treatment can also induce OS, and the prolonged use of valproic acid, widely used to treat this condition in children and adults, has been linked to impaired fertility. In one study, valproic acid-induced oxidative damage in rats testis and epididymis decreased the antioxidant capacity and, consequently, reduced sperm motility and vigor (Ourique et al., 2016). Moreover, resveratrol prevented the OS adverse effects of prolonged use of valproic acid (Ourique et al., 2016). Finally, antibiotics may also be responsible for decreasing male fertility potential by inducing OS conditions. Metronidazole is a drug that causes a transitory male infertility. Al-Alami, Shraideh and Taha (2017) showed that this effect could be reduced using 5 mg/kg of rosmarinic acid. In this study, authors found that this natural antioxidant improved testicular ultrastructure and sperm parameters like morphology, sperm count and motility (Al-Alami et al., 2017).

4.2.4 Physical Exercise

Some exercise can affect testis in different ways, mainly because of thermal conditions that induce OS and thus impair spermatogenesis. Intense exercise increases oxygen consumption, leading to an overproduction of free radicals. Nevertheless, in a protocol to study the OS caused by high-intensity swimming in a rat model, no negative effect on spermatogenesis was detected when evaluated by a histological approach (Kalantari et al., 2017). One possible explanation for this result is that the effects of intense exercise on spermatogenesis might be associated with time and exercise load, hypothesis reinforced by a study published by Moayeri and colleagues (2017) in which opposite outcomes were found (Moayeri et al., 2018).

In Kalantari and colleagues (2017) study, rats were submitted to chronic aerobic swimming for five weeks. They were distributed in the following four groups: control group (no swimming), swimming, swimming previously treated with 50 mg/kg of vitamin E, and sham group (treated with saline and exposed to water but without swimming). In this study, the authors found that the antioxidant capacity, measured by ferric reducing antioxidant power (FRAP) assay, of rats that were submitted to swimming was higher than the control group. Moreover, the MDA level was not different from control when aerobic swimming was induced, although this level was lower in the vitamin E group. Finally, spermatogenesis quality was higher in the group treated with vitamin E compared to the control group. However, these results may be inaccurate for being measured 48 hours after the last exposure to swimming (Kalantari et al., 2017), overlooking the period of complete spermatogenesis. In Moayeri and colleagues (2017) study, no information regarding how long after the last exposure to swimming the rats were euthanized. However, in this study, the authors found that melatonin (10 mg/kg) partially restored the decrease in seminal vesicle weight, motility and sperm morphology of animals submitted to swimming. When apoptosis of testicular germ cells was assessed by the TUNEL assay, an increment in germ cell death in groups exposed to swimming was observed, with partial improvement in the melatonin-treated group. Moreover, swimming negatively impacted the antioxidant system, and melatonin did not restore GPx, CAT, SOD and GR (glutathione reductase) values (Moayeri et al., 2018).

Finally, in Arun and colleagues (2018) study, rats were submitted to chronic stress by forced immobilization followed by swimming in cold water. Although the time of exposure to forced swimming was short (15 min/day) comparing to previous studies, it was enough to cause a critical reduction in the absolute weight of the testis, testosterone levels, and sperm concentration, in addition to an increase in sperm abnormalities and MDA levels. Moreover, when 50 mg/kg/day of leaf extract of *Phyllanthus emblica* (PE) was used, most of these characteristics were improved in chronically stressed rats. Chronic stress altered tyrosine phosphoproteins of the testis, which also was partially restored by PE (Arun et al., 2018).

4.2.5 Environmental exposure

Men are exposed to several conditions that contribute to OS and therefore may reduce male fertility potential. Environmental stressors are known to decrease the male fertility potential in an idiopathic manner. Bisphenol A (BPA), a molecule present in plastic packaging, is one of the most studied environmental stressors, being considered as a public health concern since it is one of the chemical compounds of endocrine disruption and can alter the physiological status. Concerning the reproductive system specifically, evidence shows that BPA alters testicular function (Quan et al., 2017) for its estrogen-like activity and for negatively interfering on spermatogenesis (G.-L. Zhang et al., 2013). In addition, it can alter sperm quality, as seen in rats, and participates in a rupture of the hypothalamic-pituitary-gonadal axis (Wisniewski et al., 2015).

The BPA impact on testicular OS may be caused by the generation of free radicals (Khalaf et al., 2019), although this mechanism is still unclear. An increase in the apoptosis rate in epididymis sperm was observed in rats treated orally with BPA (Srivastava & Gupta, 2018), as well as a decrease in testosterone levels, testicular weight and sperm concentration. However, this toxic effect on reproductive parameters was mitigated when rats exposed to BPA were simultaneously treated with vitamin E. Selenium and nano-selenium have also been used to protecting against the toxic effects of BPA (Khalaf et al., 2019), showing remarkable effects. In addition, the use of nano-selenium alone increased the level of CAT compared to control and, when animals exposed to BPA were treated with nano-selenium, a higher effect was observed in decreasing sperm DNA fragmentation (Khalaf et al., 2019).

Polyvinyl chloride (PVC), another compound used in the plastic industry, is also related to toxic effects on the reproductive system. However, since it is mostly used in the construction area, it offers less extensive contamination than BPA. Wistar rats administrated orally for 60 days to PVC showed a significant reduction in the weight of the reproductive organs, with a decrease in steroidogenic activity and, consequently, a reduction in sperm count and sperm motility (Archana et al., 2018). In addition, low antioxidant levels (SOD, CAT) and high levels of LPO evidenced oxidative stress in these animals. Nonetheless, simultaneous treatment of these animals with resveratrol had an important protective effect on steroidogenesis and spermatogenesis, demonstrated by better architecture of the seminiferous tubules and better reproductive performance.

An important source of environmental exposure is the contamination of the soil and plantations with herbicides, fungicides, or insecticides, among which arsenic is a threatening component. Reproductive problems associated with arsenic exposure seem to occur as a consequence of excessive ROS formation, along with decreased testosterone synthesis (Prathima et al., 2018). However, in rats, vitamin C or zinc chloride administration contributed to decreased sperm defects (Altoé et al., 2017). Another promising antioxidant against arsenic is the lipoic acid, a potent free radical scavenger. In another study with rats, lipoic acid reverted the OS caused by arsenic, increasing total sperm count, sperm viability and motility, and testosterone levels (Prathima et al., 2018).

Using an African plant, *Chasmanthera dependens*, Quadri and Yakubu (2017) found a promising result concerning the seminal and fertility aspects of rats submitted to exposure to sodium arsenite. On the other hand, despite this positive result, animals exhibited some degree of toxicity, demonstrating the importance of conducting studies in animal models to ensure the safety of treatments (Quadri & Yakubu, 2017).

Some fungicides used in agriculture were already linked to male subfertility; carbendazim is one of them (Chenniappan & Murugan, 2017). Rats treated with this substance had a significant reduction in sperm vitality and morphology, as well as in the levels of antioxidant enzymes (CAT and SOD) and increase in MDA levels (Chenniappan & Murugan, 2017). In this study, the toxic effects of the fungicide were reversed by using the extract of *Ionidium suffruticosum* leaves, a plant of the *Violaceae* family. In the same way as fungicides, fungi can also generate OS: aflatoxins produced by fungi of the genus *Aspergillus* are associated with infertility (Cao et al., 2017). Moreover, this study showed that the use of selenium (0.4 mg/kg) can partially reverse the effects of B1 Aflatoxin on sperm parameters (motility, concentration, and morphology), as well as on testosterone, ROS, MDA levels and GSH activity. A possible mechanism for the restorative effect of selenium in the low testosterone levels induced by aflatoxin, is the increased expression of the steroidogenic enzymes StAR, P450scc and 17β-HSD (Cao et al., 2017).

The toxicity of lead compounds is another way of inducing oxidative stress. The effects of lead acetate, recognized for contributing to testicular degeneration, were mitigated by pulicalagin, a polyphenol with antioxidant properties (Rao et al., 2016). In this study, alterations in LPO and GSH levels, with improvement in sperm characteristics, were related to the activation of nuclear factor erythroid-2 related factor 2-like 2 (Nrf2).

Exposure to acrylamide represents a less frequent way to induce infertility due to OS (Katen et al., 2016). Another example is the exposure to γ-rays radiation. In this case, however, the use of silymarin displays a radioprotective effect regarding sperm viability preservation (Fatehi et al., 2018). Finally, the air pollution caused by industries and motor vehicles is a factor to which the whole population is exposed. Particulate matters, that are amongst the main components of air pollution, can impact spermatogenesis by MDA accumulation, reducing sperm concentration and increasing sperm abnormalities. In addition, the effects on spermatogenesis seem to be related to ROS-mediated MAPK pathways, and the use of vitamins C and E combined may mitigate these toxic effects (B. Liu et al., 2019).

4.2.6 Heat stress

In mammals, the testis temperature needs to be between 2 - 6 °C lower than the body temperature to properly perform spermatogenesis. Testicular heating may increase the production of ROS in several mammal species like rodents and men, although this is not applicable, for example, to marine mammals that have the testes within the abdominal cavity. Thermoregulation of the testis is maintained by complementary mechanisms, such as the presence of sweat glands in the scrotum, the cremaster muscle that approximates or moves the testicles away from the body, and the juxtaposition of the testicular veins forming a tangle around the testicular artery, the so-called pampiniform plexus, responsible for cooling the blood that reaches the testes. These mechanisms may be disturbed by various factors, like environmental exposures to high temperatures or clinical conditions such as cryptorchidism and varicocele.

Several natural or artificial antioxidant components have been tested, initially in animal models, to treat male infertility caused by heat stress-induced OS. Among the results of this review, there is even a formulation with an undeclared composition – probably for patent reasons – that was considered promising (Gharagozloo et al., 2016). The study used knockout mice for the enzyme GPx5, who, for this reason, experienced testicular thermal stress. The treatment decreased levels of DNA damage in sperm and re-established the pregnancy rate to almost normal.

The use of plant extracts to improve seminal aspects associated to OS caused by heat is noteworthy. Due to their antioxidant characteristics, these extracts are widely used in traditional medicine. Here we discuss the importance of three extracts, that were previously used in heat-induced rodents (Kim et al., 2017; Kumar Roy et al., 2016; Ngoula et al., 2017). In the first report, methanol extract of *Mallotus roxburghianus* was used to test whether the harmful effects of scrotal hyperthermia in rats could be reversed (Kumar Roy et al., 2016). The heat injury markedly increased MDA levels and reduced

antioxidant capacity and testosterone concentrations. Moreover, the testicular histology showed a disarrangement of the seminiferous tubules, blocking spermatogenesis. *M. roxburghianus* administration for 14 days after heat induction, improved the testicular function by restoring the antioxidant capacity and testosterone levels. Moreover, the treatment increased cell proliferation (Kumar Roy et al., 2016). A beneficial effect was also found using the oil extracted from guava (*Psidium guajava*) leaves in heat-stressed cavies. Oral administration (100 µL/kg body weight) increased SOD activity and decreased the levels of NO and MDA comparing to non-treated animals exposed to 35 or 45 ºC (Ngoula et al., 2017). Authors also found that this treatment enhanced the sperm concentration of cauda epididymis and individual motility, becoming similar to control (not exposed to heat).

Panax ginseng has been largely used in Asian countries to improve health conditions like diabetes, hypertension, cancer, acquired immune deficiency syndrome, and sexual dysfunction. In a recent study, ginseng showed a protective effect on sperm kinematic values in heat-stressed male rats (Kim et al., 2017), partially or totally reverting the reduction caused by heat-stress in the expression of transcripts and proteins of hormonal receptors (FSH, LH, and androgen receptors) of the testis. Ginseng also reverted alterations in the proteomic profile of GPx4 and GSTM5 antioxidant enzymes, along with the alterations caused to CREB1 and INHA, two proteins that are important for spermatogenesis (Kim et al., 2017).

4.3. Limitations

Studies with humans had several distinct outcomes of interest, with sample sizes varying from 10 to more than 300 people. Moreover, sperm analyzes were carried out using the WHO guidelines of 1999 and 2010, with different populations being analyzed. Experimental studies with animal models to elucidate mechanisms underlying male infertility, in general, had more controlled methodological conditions. However, multiple methods have been applied to induce testicular OS, which hinders results comparison. In addition, sperm aspects and parameters were accessed in non-standard manners, since there is no reference manual (such as the WHO) for examining and processing laboratory samples.

5. Conclusion

Understanding oxidative balance and studying the function and application of antioxidants in oxidized systems is undoubtedly important. The use of natural antioxidants both oral and *in vitro* supplementation in alternative medicine for reproductive purposes is increasing in an attempt to achieve better gametes and embryos. The supplementation effectiveness will depend on both the couple infertility condition and the management choice for male factor infertility treatment. Vitamins C, B and E, selenium and zinc are the most commonly used antioxidants, with remarkable evidences in improving seminal pathophysiological conditions. However, despite all the available evidence, this area still lacks more assertive and conclusive answers, since these substances' mechanisms are not fully understood. The use of experimental models is one useful tool to identify these mechanisms under controlled environments.

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