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A Global Survey of Reproductive Specialists to Determine the Clinical Utility of Oxidative Stress Testing and Antioxidant Use in Male Infertility

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Purpose: The use of antioxidants is common practice in the management of infertile patients. However, there are no established guidelines by professional societies on antioxidant use for male infertility.

Materials and Methods: Using an online survey, this study aimed to evaluate the practice pattern of reproductive specialists to determine the clinical utility of oxidative stress (OS) testing and antioxidant prescriptions to treat male infertility.

Results: Responses from 1,327 participants representing 6 continents, showed the largest participant representation being from Asia (46.8%). The majority of participants were attending physicians (59.6%), with 61.3% having more than 10 years of experience in the field of male infertility. Approximately two-thirds of clinicians (65.7%) participated in this survey did not order any diagnostic tests for OS. Sperm DNA fragmentation was the most common infertility test beyond a semen analysis that was prescribed to study oxidative stress-related dysfunctions (53.4%). OS was mainly tested in the presence of lifestyle risk factors (24.6%) or sperm abnormalities (16.3%). Interestingly, antioxidants were prescribed by 85.6% of clinicians, for a duration of 3 (43.7%) or 3–6 months (38.6%). A large variety of antioxidants and dietary supplements were prescribed, and scientific evidence were mostly considered to be modest to support their clinical use. Results were not influenced by the physician's age, geographic origin, experience or training in male infertility.

Conclusions: This study is the largest online survey performed to date on this topic and demonstrates 1) a worldwide understanding of the importance of this therapeutic option, and 2) a widely prevalent use of antioxidants to treat male infertility. Finally, the necessity of evidence-based clinical practice guidelines from professional societies is highlighted.

Keywords: Antioxidants; Male infertility; Oxidative stress; Reproduction, physicians; Survey

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INTRODUCTION

Globally, infertility impacts 15% of couples trying to conceive. The incidence of male infertility has been increasing over the past several decades, ranging from 20% to 70% worldwide [1,2]. The male partner is found to be solely responsible in 20%–30% of cases, and contributes to couple infertility in approximately 50% of cases [3]. The etiologies and risk factors for male infertility are diverse. In addition to genetic causes, common acquired causes include varicocele, reproductive tract infections/inflammation, endocrine abnormalities, cryptorchidism, medications, malignancy, radiation and chemical or chemotherapy exposure, environmental

and lifestyle factors, and underlying medical comorbidities [4]. Despite the wide spectrum of potential causes, a large proportion of male infertility cases remain as unexplained male infertility (UMI) and/or idiopathic male infertility (IMI) when controlling for known female factors. In this context, oxidative stress (OS) has been established as an important etiology and/or common mechanism in many known and unknown causes of male infertility [5,6].

OS occurs when there is an imbalance between reactive oxygen species (ROS) and antioxidants (AOX), resulting in sperm DNA fragmentation (SDF) and semen abnormalities [7]. The negative impact of OS on male infertility is supported by abnormal levels of seminal oxidation-reduction potential (ORP) in up to 80% of males with IMI, a condition termed as male OS infertility (MOSI) [6]. Elevated OS can negatively impact fertility through various pathways. OS can induce the formation of mutagenic or genotoxic by-products in germ cells and spermatozoa that may result in a negative impact on spermatogenesis, semen parameters, semen quality, fertilization, pregnancy, and health consequences for future progeny [8,9]. Therefore, male fertility potential cannot be fully evaluated unless seminal OS is included in the clinical assessment.

As OS is such a prominent cause or mechanism of male infertility, the utilization of AOX to reduce seminal ROS is an important therapeutic option to improve semen parameters and fertility. Due to their widespread availability, safety profile, and low cost compared to assisted reproduction, AOX and supplements are a simple starting point for many infertile couples seeking to improve their chances of conceiving [10]. However, although considered low cost, AOX for male infertility are part of a large economic market for dietary supplements, estimated at USD 123.28 billion in 2019, with a forecasted growth rate of 8.2% annually until 2027 [11].

Commonly used AOX for male infertility include vitamins A, C, and E, L-carnitines, N-acetyl cysteine, and Co-enzyme Q10, along with important AOX co-factors zinc, selenium, and folic acid. These and numerous others are included in various registered AOX formulations [12-14] and food enriched with natural AOX [15,16]. Multiple studies have shown the benefit of AOX supplementation [17-19], and a recent Cochrane review found that oral AOX therapy may improve semen parameters and the likelihood of pregnancy [20]. However, the outcomes of clinical trials on the use of AOX in male infertility are not consistent, ranging from clear benefit [21] to no clinical effect [22], to even having significant detrimental effects [23,24]. These conflicting findings may be due to reductive stress from improper dosing of AOX or from poor study design [25]. Regardless, these studies have caused confusion among patients and clinicians alike.

While the prescription of AOX as a therapeutic option is gaining increasing attention, the lack of clinical practice guidelines endorsed by professional associations strictly limits the standardization of clinical protocols [26,27]. Even though the topic has been investigated in a large number of publications [27], there is still no consensus on regimen, dosing, or length of AOX treatment. The dose and nature of AOX prescribed is variable and determined by the local availability of AOX products and the experience of the individual practitioner. Similarly, the length of treatment reportedly varies between less than 2 months and up to 6 months [27]. However, the global practice of recommending AOX for infertile men by registered practitioners in reproductive medicine is unclear. Therefore, this study aimed to conduct a comprehensive survey of reproductive specialists to determine the pattern of using OS tests and AOX prescriptions in male infertility based on the clinical experience of professionals in the field of infertility from around the world.

MATERIALS AND METHODS

1. Ethical statement

This global online survey on male infertility, OS testing and AOX use was approved by the Institutional Review Board (IRB) of Cleveland Clinic (IRB No. 20-855).

2. Survey design and participants

This cross-sectional observational study used an internet survey as a data collection tool. The survey was designed to investigate the use of OS testing, AOX utilization and practice patterns of reproductive specialists involved in the care of patients with male infertility. A panel was formed consisting of 58 experts actively involved in the management of male infertility patients from 28 different countries, which included clinicians, researchers and scientists involved in the field of OS, AOX, and male infertility. The sur-

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vey questions were carefully drafted, reviewed, and approved by these experts. SelectSurvey (https://www.classapps.com/product_ssv5.aspx), a secured tool approved by the Cleveland Clinic's Information Technology Department, was used to populate the survey questions online. The survey was kept open for 3 months (August 10th, 2020 to November 9th, 2020) for the participants to provide their response. During this time period, the online survey link was shared with the members of various professional societies and committees (as listed in the Acknowledgement section). The targeted participants of the survey were medical professionals working in the field of reproductive medicine and those involved in the treatment of infertile couples. Incomplete or duplicate responses were excluded from the analysis.

3. Questionnaire design

The online survey was written in English and composed of 29 questions that were structured into four different sections (Supplement File). The first section (4 questions) was related to the demographics of the participants; the second section (6 questions) was regarding the profession and clinical practice of the respondents; the third section (4 questions) was on OS testing in clinical practice; and the final section (15 questions) was related to the use of AOX in clinical practice.

4. Statistical analysis of data

Survey responses were downloaded in a comma-separated values (CSV) file format from the SelectSurvey application into a secured storage device approved by the Cleveland Clinic. Descriptive statistics was conducted using MedCalc Software (version 19.0.5; MedCalc Software, Ostend, Belgium). Data was reported as the number of participants and percentage of the total who answered each survey section or relevant question. However, participant numbers might be slightly different for some optional questions. For other questions, participants could mark more than one option, hence the results were reported as the percentage calculated based on the total number of participants who answered. Results were analysed using chi-square test to examine the international trends of OS testing and AOX use by participants.

Table 1. Classification of survey participants according to demographics and professional details

SN	Classification variable	Self-reported responses
A	Geographical origin	
	Asia	621 (46.8)
	South America	262 (19.7)
	Europe	208 (15.7)
	Africa	150 (11.3)
	North America	57 (4.3)
	Australia	29 (2.2)
	Total	1,327 (100)
B	Age of participants (y)	
	25–34	201 (15.1)
	35–44	467 (35.2)
	45–54	314 (23.7)
	55–64	213 (16.1)
	≥65	132 (9.9)
	Total	1,327 (100)
C	Affiliation	
	Medical education company	164 (12.8)
	Pharmaceutical or dietary supplement company	22 (1.7)
	Market research company/advertising agency	10 (0.8)
	I am affiliated with MORE than one of the bodies mentioned in the table	17 (1.3)
	Other	68 (5.3)
	I am NOT affiliated with any of the bodies mentioned in the table	1,000 (78.1)
	Total	1,281 (100)
D	Nature of the employment	
	Attending physicians	780 (59.6)
	Advanced practice provider (PA/NP)	210 (16.0)
	Resident	83 (6.3)
	Fellow	75 (5.7)
	Academic staff	46 (3.5)
	Embryologist	20 (1.5)
	Other	19 (1.5)
	Urologist	19 (1.5)
	Gynecologist	14 (1.1)
	Andrologist	10 (0.8)
	Laboratory director	7 (0.5)
	Researcher	6 (0.5)
	Fertility specialist	5 (0.4)
	PhD student	5 (0.4)
	Student	4 (0.3)
	Biologist	3 (0.2)
	Clinic director	3 (0.2)
	Total	1,309 (100)

RESULTS

1. Survey participants

After excluding duplicate or incomplete responses (those who did not continue with questions listed in section 2, n=150) from the analysis, a total of 1,327 participants from 88 countries completed the online survey (Table 1). Not all the questions in each section were completed by each participant, as some were optional. The number of responses for each subsection is provided in Table 1. Geographical distribution of the participants included Asia (46.8%), South America (19.7%), Europe (15.7%), Africa (11.3%), North America (4.3%) and Australia (2.2%). The majority of participants were aged 35–64 years old (75.0%), whereas the remaining were aged 25–34 years old (15.1%) or more than 65 years old (9.9%).

A total of 1,281 (96.5%) participants responded to the self-reported nature of employment. Here, participants were identified primarily as attending physicians (59.6%) and advanced practice providers such as physician assistants and nurse practitioners (16.0%), alongside resident (6.3%) or fellows (5.7%) and academic staff (3.5%). A total of 1,323 (99.7%) participants responded to the primary work setting, which was most prominently reported as private practice (35.7%) or academic hospitals/clinics (26.9%). Out of 1,325 (99.8%) participants who responded to the question about their profession, nearly half (49.9%) reported themselves to be involved in more than one of the registered professions listed, while the most frequent self-reported single registered profession was in the field of urology (20.2%), andrology (8.8%) and gynecology (6.7%). The full cohort (n=1,327) responded to the questions about years of experience and specific training in male infertility. Here, most of these participants reported clinical practice experience of more than 10 years (61.3%), with special training in the field of male infertility either as part of their residency (47.5%) or fellowship (29.2%) training.

Table 1. Continued

SN	Classification variable	Self-reported responses
E	Primary practice setting	
	Private practice or clinic	472 (35.6)
	Academic hospital or clinic	356 (26.9)
	Public hospital or clinic	93 (7.0)
	Non-academic private hospital or clinic	59 (4.5)
	Other	14 (1.1)
	Non-teaching community hospital	6 (0.5)
	More than one of the mentioned primary setting	323 (24.4)
	Total	1,323 (100)
F	Registered profession	
	Urology	268 (20.2)
	Andrology	116 (8.8)
	Gynecology	89 (6.7)
	Reproductive specialist	88 (6.6)
	Other	34 (2.6)
	Dermatology	17 (1.3)
	Embryology	15 (1.1)
	General practitioner	14 (1.1)
	Obstetrics	12 (0.9)
	Researcher	11 (0.8)
	More than one of the mentioned registered professions	661 (49.9)
	Total	1,325 (100)
G	Years of practicing	
	Less than 2 years	98 (7.4)
	2–5 years	179 (13.4)
	5–10 years	237 (17.9)
	More than 10 years	813 (61.3)
	Total	1,327 (100)
h	Specialty training in the field of male infertility	
	Yes, as part of my specialty training (residency)	63 (47.5)
	Yes, as a specific training program (fellowship)	387 (29.2)
	No	310 (23.3)
	Total	760 (100)

Values are presented as number (%).
Survey participants were classified according to their responses regarding their (A) geographical origin, (B) age, (C) affiliation, (D) nature of the employment, (E) primary practice settings, (F) registered profession, (G) years of practice, and (H) specialty training in male infertility.
SN: serial number, PA/NP: physician assistant/nurse practitioners.

2. Oxidative stress testing

A total of 1,305 (98.3%) participants responded to the use of OS testing as part of male fertility evaluation, where 34.3% reported the use of OS testing in their clinical practice (Table 2). A sub-analysis was con-

Table 2. Participants (n=1,305) response to the use of oxidative stress-related biomarkers in the clinical evaluation of male infertility

Response	Oxidative stress testing as a part of the male fertility evaluation
Yes	448 (34.3)
No	857 (65.7)

Values are presented as number (%).

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ducted to investigate the most commonly used assays to assess OS. Among the tests presented in Fig. 1, SDF (53.4%) was the most commonly utilized, followed by ORP (6.3%). Moreover, 31.3% reported using more than one assay to evaluate OS in infertile men (Fig. 1).

A total of 416/448 (92.9%) participants responded to the clinical conditions for OS testing. Survey results revealed that 59% (n=249) of clinicians recommended OS testing for patients with abnormal semen parameters and 55.8% of clinicians ordered OS testing for patients with UMI (n=232) (Fig. 2A). Survey participants reported that patients with risk factors such as smoking, alcohol consumption or drug abuse/misuse (n=308, 74.0%) and advanced paternal age (n=275, 66.1%) were most indicated for lifestyle-related OS testing (Fig. 2B).

3. Indication for antioxidant therapy

A total of 1,260 (94.9%) responded to the question on the prescription of AOX for the treatment of male infertility. Here, a high percentage (n=1,078, 85.6%) of participants recommend AOX as a therapeutic option in the management of male infertility, either routinely (n=550, 43.7%) or for specific groups of patients (n=528, 41.9%) (Table 3: a). Of those participants who did not use OS testing as a part of male infertility evaluation (n=857), a high percentage (n=679, 79.2%) reported prescribing AOX treatment.

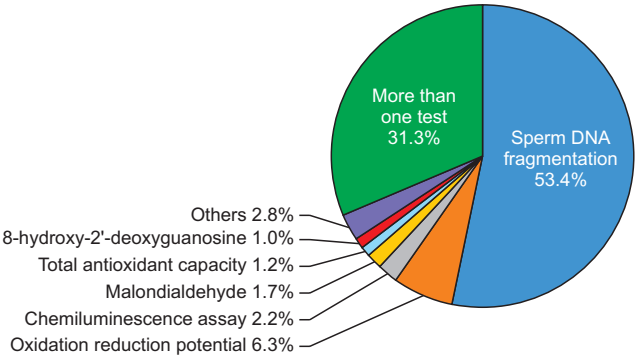

Fig. 1. Tests of oxidative stress that are commonly ordered by participants in the evaluation of male infertility.

A total of 1,039 out of 1,078 (96.4%) responded to the multiple option question on the clinical conditions for which AOX-based therapy was prescribed. Here, the most common clinical conditions for which AOX-based therapy were prescribed were risk factors for OS (such as obesity, age, smoking) (n=707, 68.0%), idiopathic oligoasthenoteratozoospermia (n=659, 63.4%), UMI (n=651, 62.6%), isolated asthenozoospermia (n=629, 60.5%) and teratozoospermia (n=501, 48.2%) (Table 3: b).

A total of 1,039 participants responded to the question on the duration of AOX treatment of male infertility. Here, the majority of the participants recommend AOX treatment for a duration of 3 months (n=454, 43.7%) or up to six months (n=401, 38.6%) (Table 3: c).

Furthermore, the survey results revealed that 584 participants out of 1,039 (56.2%) who responded to this question treat 25%–75% of their patients with AOX (Fig. 3). A total of 1,039 participants responded to the question on the preference of AOX used in clinical practice. Here, the most commonly prescribed individual AOX were zinc (n=753, 70.9%), vitamin E (n=717, n=69.0%), L-carnitine (n=706, 67.9%) and Co-enzyme Q10 (n=675, 65.0%) (Table 4).

A total of 708 participants were familiar with dietary AOX supplements, while 688 (97.2%) responded to the question on the top 10 most common commercial

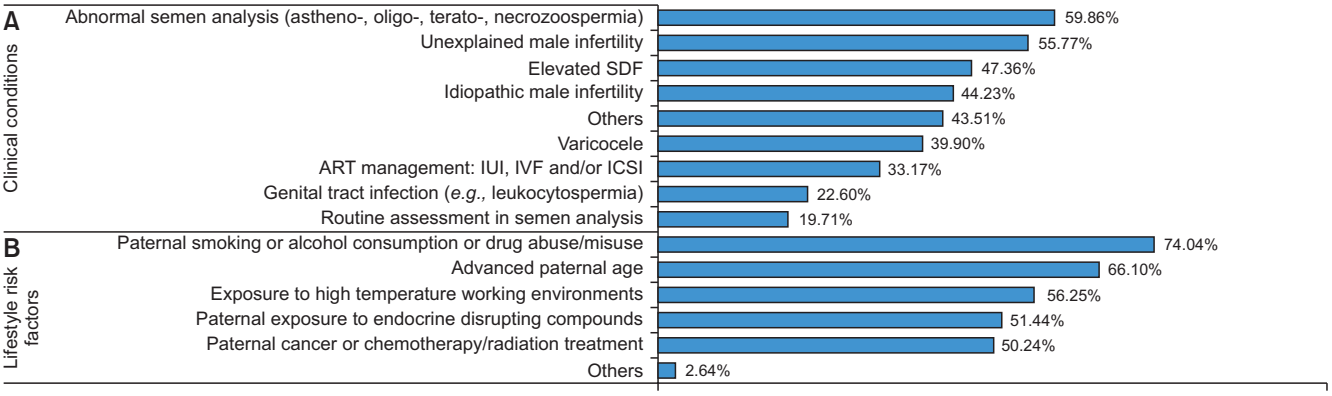

Fig. 2. Indication for oxidative stress testing based on (A) clinical conditions and (B) lifestyle risk factors. SDF: sperm DNA fragmentation, ART: assisted reproduction techniques, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection.

Table 3. Indications for AOX therapy

SN	Indications for AOX therapy	Self-reported responses
A (n=1,260)	Prescription of the antioxidants for the treatment of male infertility	
	No, I never do so	182 (14.4)
	Yes, but only for specific groups of patients	528 (41.9)
	Yes, I routinely do so	550 (43.7)
	Total	1,260 (100)
B (n=1,039)	Clinical conditions treated	
	Risk factors for oxidative stress (obesity, age, smoking)	707 (68.0)
	Idiopathic oligoasthenoteratozoospermia	659 (63.4)
	Unexplained infertility	651 (62.6)
	Isolated asthenozoospermia	629 (60.5)
	Isolated teratozoospermia	501 (48.2)
	Isolated oligozoospermia	479 (46.1)
	Varicocele	429 (41.3)
	Increased SDF	377 (36.3)
	Empirically before assisted conception	536 (51.6)
	Necrozoospermia	287 (27.6)
	Azoospermia	166 (16.0)
	Other	11 (1.1)
	Altered seminal oxidative stress markers	7 (0.7)
	Genital tract infection/inflammation	7 (0.7)
C (n=1,039)	Duration of antioxidant treatment (mo)	
	<3	83 (8.0)
	3	454 (43.7)
	3–6	401 (38.6)
	6–9	27 (2.6)
	Indefinitely, until achieving conception	74 (7.1)

Values are presented as number (%).
(A) Prescription of AOX in clinical management of male infertility, (B) clinical conditions treated (percentage calculated based on the total number of participants answering this multiple option question), and (C) duration of treatment.
SN: serial number, AOX: antioxidant, SDF: sperm DNA fragmentation.

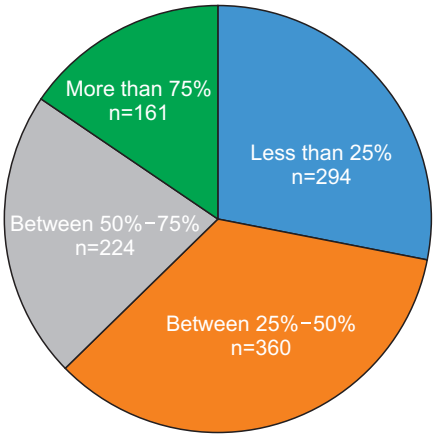

Fig. 3. Percentage of patients recommended for AOX treatment by the participants to the survey. AOX: antioxidant.

dietary AOX supplements (Table 5). While 177 participants did not recommend the AOX treatment, 1,039 participants recommended it mostly based on the scientific evidence (n=718, 69.1%) and personal experience (n=616, 59.3%) (Table 6).

Participants testing for seminal OS and prescribing AOX, respectively, were further analyzed based on their age, geographical origin, experience and the training in the field of male infertility. The participants using the seminal OS markers during evaluation of male infertility were not influenced by their age or years of experience in the field (Table 7: a). Conversely, the geographic origin significantly influenced this decision ($p<0.001$), with 53.2% of respondents from Asia opting for no testing (Fig. 4). Participants who had received a specific training in male infertility were more

Table 4. Most commonly prescribed individual antioxidants for the treatment of male infertility

Antioxidants	Self-reported responses
Zinc	737 (70.9)
Vitamin E	717 (69.0)
L-carnitine	706 (67.9)
Co-enzyme Q10	675 (65.0)
Vitamin C	604 (58.1)
Selenium	566 (54.5)
Folic acid (vitamin B9)	437 (42.1)
L-arginine	422 (40.6)
Vitamin D	309 (29.8)
N-acetyl cysteine	271 (26.1)
Combination of vitamins with carnitine	266 (25.6)
Vitamin A or relevant carotenes	242 (23.3)
Combination of vitamins C and E	221 (21.3)
Lycopene	220 (21.2)
Methylcobalamin (vitamin B12)	161 (15.5)
Riboflavin (vitamin B2)	149 (14.3)
Thiamine (vitamin B1)	147 (14.1)
Glutathione	140 (13.5)
Acetyl carnitine	138 (13.3)
Herbal antioxidants or products	128 (12.3)
Pentoxifylline	128 (12.3)
Pyridoxal-5-phosphate (vitamin B6)	119 (11.4)
Methylfolate	104 (10.0)
Biotin	102 (9.8)
Alpha-lipoic acid	79 (7.6)
Docosahexanoic acid (DHA)	77 (7.4)
Pantothenic acid (vitamin B5)	67 (6.4)
Vitamin B3 (niagen)	61 (5.9)
Melatonin	53 (5.1)
L-tyrosine	48 (4.6)
Other	41 (3.9)
Vitamin K2	29 (2.8)
Chelated copper	22 (2.1)
Para-aminobenzoic acid (PABA)	22 (2.1)
Methylsulfonylmethane (MSM)	17 (1.6)
Phosphatidylcholine	15 (1.4)

Values are presented as number (%*).
*Percentage was calculated based on the total number of participants (n=1,039) who answered this multiple option question.

likely to order OS testing (n=378 out of 448), although interestingly the majority of participants chose no testing (n=626 out of 857) (p<0.001) (Fig. 4). With respect to prescribing AOX, the percentage of participants who recommended AOX was significantly related to the participant's age (p<0.001), geographic origin (p<0.004), experience or training in male infertility (p<0.001) (Table 7: b, Fig. 5).

The perception of AOX treatment in clinical practice from the participants point of view

A total of 1,152 participants out of 1,327 (86.8%) responded to the question on the strength of the evidence for AOX use in male infertility. Here, more than half of the participants considered the evidence supporting AOX use in clinical practice as modest (52.3%), that is a score of four in a scale where 5 was strong, while the remaining practitioners were divided between "the evidence supporting its use is strong" (19.7%) and "no good evidence supporting its use" (22.2%) (Table 8).

A total of 1,172 out of 1,327 (88.3%) participants responded to the multiple response question on the limitations of AOX use. Here, the most important reasons for limiting its use were cost (n=611, 52.1%), duration of treatment which could postpone other treatments (such as assisted reproduction techniques [ART]) (n=528, 45.0%), and low adherence of patients to the therapy (n=323, 27.6%).

A total of 1,172 out of 1,327 (88.3%) participants responded to the multiple response question on the most appropriate outcomes to be evaluated after AOX treatment. Here, survey results indicated standard semen parameters (n=648, 55.3%), live birth rate (n=644, 54.9%), SDF (n=576, 49.1%) and clinical/ongoing pregnancy rate (n=555, 47.3%) as possible endpoints for the evaluation of AOX efficacy.

A total of 1,172 out of 1,327 (88.3%) participants responded to the multiple response question on the development of clinical guidelines. This was favoured by most of the participants (96.3%), who considered it either as much needed (n=607, 51.8%) and/or helpful (n=521, 44.5%) (Table 8).

DISCUSSION

AOX therapy has long been considered as an appealing treatment modality for male infertility [28]. The reasons for the interest and use of AOX are multiple. AOX supplements are easily accessible as over the counter products, are relatively inexpensive, and have few side effects. Importantly, they can potentially reverse seminal OS which is believed to be a common pathophysiology linking multiple etiologies with male

Table 5. Top 10 most common commercial dietary AOX supplements recommended for treatment of male infertility

Product	Company	City, Country	Value
Proxeed Plus	Alfasigma	Milan, Italy	254 (36.9)
Profertil	LENUS Pharma GesmbH	Vienna, Austria	179 (26.0)
Fertilaid	Fairhaven Health	Bellingham, WA, USA	135 (19.6)
Fertilix	Fertilix	Hasbrouck Heights, NJ, USA	106 (15.4)
Conception Men	Eu Natural	Henderson, NV, USA	61 (8.9)
Orthomol Plus	Orthomol	Langenfeld, Germany	56 (8.1)
Conceive Plus	Sasmar Inc.	Chicago, IL, USA	55 (8.0)
FH Pro for Men	Fairhaven Health	Bellingham, WA, USA	40 (5.8)
Coast Science	Coast Science	San Diego, CA, USA	39 (5.7)
Conception XR	Theralogix	Rockville, MD, USA	34 (4.9)

Values are presented as number (%*).
AOX: antioxidants.
*Percentage was calculated based on the total number of participants (n=688) who answered this multiple option question.

Table 6. Reasons selected by clinicians to recommend (n=1,039) or not recommend (n=177) the use of AOX in treatment of male infertility

Reasons to recommend	Self-reported responses	Reasons not to recommend	Self-reported responses
Scientific evidence	718 (69.1)	Lack of scientific evidence	85 (48.0)
Personal experience	616 (59.3)	Unfamiliar with the field	76 (42.9)
Relevant knowledge	271 (26.1)	Personal experience	14 (7.9)
Advice from peers	235 (22.6)	Other	23 (13.0)
Trained by someone that uses them	222 (21.4)		
Patient request/feedback	185 (17.8)		

Values are presented as number (%*).
AOX: antioxidants.
*Percentage was calculated based on the total number of participants who answered these multiple option questions.

infertility [7]. However, there is little information available on the use of OS testing and AOX in clinical practice.

1. Oxidative stress testing in clinical practice

The survey results show that there is a relatively low proportion of participants conducting OS testing. This may be due to lack of consensus on appropriate tests and their clinically relevant cut-off values, and an absence of standardization of laboratory techniques [29]. Although sensitivity and specificity of various tests have been published, they remain variable, non-standardized and without general diagnostic recommendations [30,31]. Furthermore, many tests for OS are expensive, may be time-consuming, often require specialized training, and are therefore not readily available in clinical and diagnostic laboratories [29]. This may be particularly apparent in developing countries, where empirical prescription of AOX in suspected cases of OS may represent a less expensive alternative to advanced testing. Interestingly, though 85.6% (Table 3: a) of participants reported the use of AOX, either routinely (43.7%) or in specific patient groups (41.9%), the majority (65.7%) (Table 2) did not use OS testing in the assessment of their patients. This may be due to the fact that the prescription of AOX is likely cheaper and more convenient compared to the assessment of OS. However, this is a potential clinical concern, as without identifying men with OS and monitoring ROS levels during therapy, there exists the risk of inducing reductive stress due to excessive AOX therapy. Reductive stress can be as damaging as OS [25,32] and may itself be a cause of male infertility [33].

Numerous testing methods have been developed over the past few decades to determine OS or measure ROS in semen [29]. However, these tests currently have limited practical use and are mostly limited to research [34]. This is reflected in the current survey results, with chemiluminescence detection being used by only 2.2% of participants (Fig. 1). Indirect tests determine the im-

pact of OS on lipids, proteins or DNA, or determination of conditions closely associated with OS, and therefore do not include direct measures of ROS or AOX. These include assays for malondialdehyde (MDA), thiobarbituric acid (TBARS) or 4-hydroxynonenal (4-HNE) as mutagenic by-products of lipid peroxidation, 8-hydroxy-2-deoxyguanosine (8-OHdG) as a marker of damage to DNA, and other assessments of SDF [5,34-36].

Table 7. Patterns of oxidative stress testing and prescription of AOX according to the participants' age, geographic origin, experience (in years) and training in male infertility

Seminal oxidative stress testing as a part of male infertility evaluation							
Testing for oxidative stress	Age of practitioner (y)						
	25–34	35–44	45–54	55–64	≥65	Total responses	p-value*
Do not test OS markers	130 (15.2)	316 (37.1)	196 (23.0)	133 (15.6)	78 (9.1)	853 (100)	NS
Do test OS markers	67 (15.0)	139 (31.0)	112 (25.1)	78 (17.5)	51 (11.4)	447 (100)	
	Geographic macro-areas						
	Africa	Asia	Australia	Europe	North America	South America	Total responses	p-value*
Do not test OS markers	108 (12.6)	454 (53.2)	13 (1.5)	111 (13.0)	41 (4.8)	127 (14.9)	854 (100)	<0.001
Do test OS markers	39 (8.7)	148 (33.1)	16 (3.6)	93 (20.8)	16 (3.6)	135 (30.2)	447 (100)	
	Years of experience						
	<2	2–5	5–10	>10	Total responses	p-value*	
Do not test OS markers	63 (7.3)	117 (13.6)	155 (18.2)	522 (60.9)	857 (100)	NS	
Do test OS markers	32 (7.1)	60 (13.4)	77 (17.2)	279 (62.3)	448 (100)		
	Specific training in male infertility						
	No training in male infertility	Yes, as a specific training program (fellowship)	Yes, as part of my specific training (residency)	Total responses	p-value*		
Do not test OS markers	231 (26.9)	216 (25.3)	410 (47.8)	857 (100)	<0.001		
Do test OS markers	70 (15.6)	171 (38.2)	207 (46.2)	448 (100)			
Prescription of antioxidants to treat male infertility							
Prescription of AOX	Age of practitioner (y)						
	25–34	35–44	45–54	55–64	≥65	Total responses	p-value*
Do not prescribe AOX	42 (23.2)	59 (32.6)	38 (21.1)	18 (9.9)	24 (13.2)	181 (100)	<0.001
Do prescribe AOX	146 (13.6)	377 (35.1)	263 (24.5)	185 (17.2)	103 (9.6)	1,074 (100)	
	Geographic macro-areas						
	Africa	Asia	Australia	Europe	North America	South America	Total responses	p-value*
Do not prescribe AOX	10 (5.5)	100 (55.2)	2 (1.2)	28 (15.5)	14 (7.7)	27 (14.9)	181 (100)	<0.004
Do prescribe AOX	129 (12.0)	480 (44.6)	27 (2.5)	171 (15.9)	41 (3.8)	227 (21.2)	1,075 (100)	
	Years of experience						
	<2	2–5	5–10	>10	Total responses	p-value*	
Do not prescribe AOX	28 (15.3)	28 (15.3)	33 (18.2)	93 (51.2)	182 (100)	<0.001	
Do prescribe AOX	64 (5.9)	139 (12.9)	190 (17.6)	685 (63.6)	1,078 (100)		
	Specific training in male infertility						
	No	Yes, as a specific training program (fellowship)	Yes, as part of my specific training (residency)	Total responses	p-value*		
Do not prescribe AOX	80 (43.9)	39 (21.5)	63 (34.6)	182 (100)	<0.0001		
Do prescribe AOX	211 (19.6)	334 (31.0)	533 (49.4)	1,078 (100)			

Values are presented as number (%).
AOX: antioxidant, OS: oxidative stress, NS: not significant.
*p-value obtained by chi-square test.

OS is a well-recognized cause of SDF [29,37]. Recently, Agarwal et al [38] proposed clinical guidelines for SDF testing and showed that there is strong evidence that varicocele, IMI, UMI and a detrimental lifestyle are associated with increased OS, and hence necessitate SDF assessment. This is supported by the survey results, where SDF assessment was most strongly reported for use as a marker of OS by 53.4% of respondents (Fig. 1). SDF has been found to correlate positively with MDA, and negatively with superoxide dismutase and glutathione peroxidase, in semen samples of infertile men [39]. This correlation has also been reported in males with subclinical, normozoospermic, asthenozoospermic and oligozoospermic varicocele [40]. Linear correlations between SDF and seminal OS with increasing paternal age [41] have also been noted. However, it is suggested that both SDF and another measure of OS should be used in the evaluation of infertile men [39]. This is supported by Homa et al [42], who suggests that neither chemiluminescence nor ORP should be used alone to determine OS. This may explain a high proportion (31.3%) of participants reporting the use of more than one assessment for OS. Although there are numerous SDF testing methods for diagnostic and research use, with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), sperm chromatin structure analysis (SCSA), and sperm chromatin dispersion (SCD) being most commonly used, these tests remain unstandardized and without clinical consensus [38,43,44].

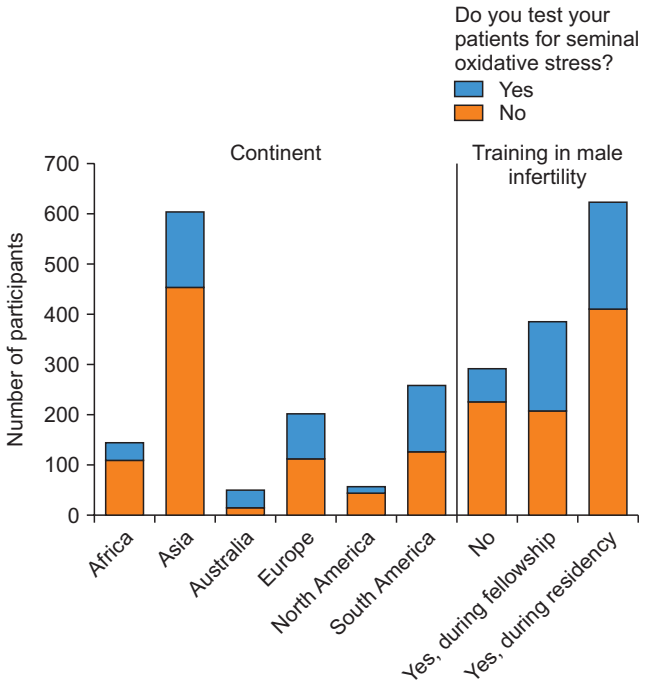

Fig. 4. Oxidative stress testing based on the geographic origin of the participants and training in male infertility.

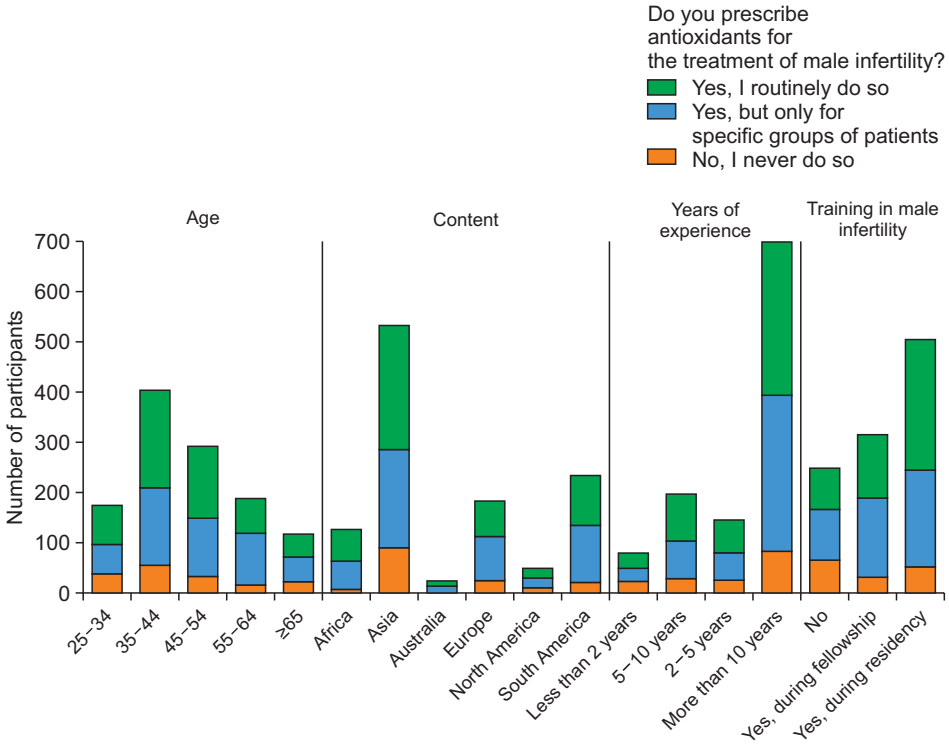

Fig. 5. Antioxidant prescription based on the age, geographic origin, experience of the participants and their training in male infertility.

Table 8. Perception of AOX treatment in clinical practice from the point of view of the participants

SN	Survey questions	Self-reported responses
A	Strength of the evidence for AOX use in male infertility (n=1,152)	
	The evidence supporting its use is modest	602 (52.3)
	There is no good evidence supporting its use	256 (22.2)
	The evidence supporting its use is strong	227 (19.7)
	Do not know or not applicable to my practice	49 (4.3)
	There is evidence against its use	18 (1.5)
B	Limitations for AOX use	
	Cost	611 (52.1)
	Long-time therapy postponing definitive treatment (*e.g.*, ART)	528 (45.0)
	Low treatment adherence	323 (27.6)
	Not effective	291 (24.8)
	Side effects	44 (3.7)
	Uncertain benefit	16 (1.4)
	Lack of good evidence supporting their use	14 (1.2)
	Other	11 (0.9)
	No good evidence supporting its benefit	7 (0.6)
	They are effective only in specific patients' subgroups	4 (0.3)
C	Endpoints to be evaluated after AOX treatment	
	Standard semen parameters	648 (55.3)
	Live birth rate	644 (54.9)
	Sperm DNA fragmentation (SDF)	576 (49.1)
	Clinical/ongoing pregnancy rate	555 (47.3)
	Reactive oxygen species (ROS)	345 (29.4)
	Miscarriage rate	278 (23.7)
	Oxidation-reduction potential (ORP)	217 (18.5)
	Total antioxidant capacity (TAC)	173 (14.7)
	Other	5 (0.4)
D	The necessity of clinical practice guidelines	
	Helpful, although more well-designed clinical trials would be needed	521 (44.5)
	Much needed, as clear guidelines on the topic are lacking	607 (51.8)
	Not necessary, as antioxidants are already proved to be effective	19 (1.6)
	Not necessary, as antioxidants are ineffective	25 (2.1)

Values are presented as number (%).
SN: serial number, AOX: antioxidant, ART: assisted reproductive technology.

The survey results show that 6.3% of participants used ORP for assessment of OS, third most frequent after SDF and combination testing (Fig. 1). The ORP has been introduced recently to determine seminal OS directly as an electrochemical measurement using the male infertility oxidative system (MiOXSYS; Aytu Bioscience, Englewood, CO, USA) [31,45]. Increased seminal ORP, indicative of OS, has been reported to correlate positively with SDF when normalized with sperm concentration and motile sperm concentration [46].

Important clinical indications for OS testing includes varicocele, genitourinary tract infections, obesity, diabetes, IMI and UMI [6,35]. The most commonly reported clinical reason for OS testing in this survey was abnormal semen parameters (59.9% of participants that assess OS, Fig. 2A). This is consistent with the literature, where asthenozoospermia, teratozoospermia, leukocytospermia and increased seminal viscosity have each been suggested as potential surrogate markers of seminal OS [47-49]. Abnormal semen parameter(s) are also reflected in the diagnosis of IMI, defined as having one or more abnormal semen parameter(s) without identifiable cause [6]. IMI was reported to be an indication for OS testing by a further 44.2% of participants

that assess OS (Fig. 2A). The condition of UMI, defined as patients being infertile despite having normal semen parameters, was the second most reported clinical indication for testing (55.8% of participants that assess OS, Fig. 2A). Importantly, OS is reported in 11%–78% of males diagnosed with UMI and 30%–80% of cases of IMI [50,51]. For the latter, the term MOSI has been introduced for males with IMI and who are positive for seminal OS [6].

Numerous lifestyle factors are known to increase the risk for male infertility and abnormal semen parameters. These include alcohol, tobacco and recreational drug use, increased heat exposure to the genitals, exposure to endocrine disrupting chemicals, and radiation [52-54]. Detrimental lifestyle factors are also associated with increased OS as a common underlying mediator of male infertility [52]. This reflects in the results of this survey in which there was a relatively even spread across these risk factors as indications for OS testing (Fig. 2B).

2. Antioxidant use in clinical practice

This survey confirms the widespread use of AOX to treat men with infertility. Almost half (43.7%) of clinicians routinely propose AOX therapy to all patients, while another 41.9% offer it to men with specific indications (Table 3: a). More than half the respondents (56.2%) stated that 25%–75% of their patients received AOX therapy (Fig. 3). Even the vast majority (80%) of those who did not test for OS or SDF still prescribed AOX therapy. There are numerous possible reasons underlying this practice. Firstly, there is no specific therapy for most men with infertility and even in cases with a correctable male factor the use of AOX therapy may be beneficial. Secondly, there is widespread availability of "over the counter" AOX both individually and/or in combination, with 55.3% of respondents being familiar with dietary AOX supplements (Table 5). Thirdly, AOX have been widely represented as having numerous health benefits with a good safety profile. Thus, both clinicians and patients perceive AOX to be safe. There is also relative ease of use, where taking AOX tablets is preferable to more invasive procedures like varicocele surgery or ART. Lastly, considering patient demographics, 70.1% of the respondents were either from Asia or South America (Fig. 4). In both these regions, there is a long history of use of natural medicines, which possibly makes this patient population more compliant with long-term empirical medical therapy.

On the other hand, the study also identified several potential inconsistencies to the use of AOX. The duration of therapy recommended was generally either for 3 months (reflecting the average length of the spermatogenesis process) or 6 months, while some give it indefinitely until pregnancy (Table 3: c). Despite relative ease of taking tablets daily, patient compliance can be a challenge when the duration of treatment is long, especially since infertile couples usually want a rapid positive result. Furthermore, this may delay the utilization of more effective therapies, such as ART, while the couple awaits the unpredictable outcome of AOX therapy. Although relatively cost-effective, AOX combinations containing compounds like L-carnitine and Co-enzyme Q10 can be expensive, and when used for a long duration, may prove to be costly for economically constrained patients. The survey further showed that there is no consensus on what the outcome measure should be to evaluate the benefits obtained from AOX therapy. While 55.3% of participants suggested improvement in semen parameters, there was an equal number who felt that the end point should be live birth rate (Table 8). Other end-points suggested were reduction in SDF or clinical pregnancy rates (Table 8). This diversity of opinion causes difficulty in conducting good clinical studies on AOX therapy that would gain widespread acceptance. Significant diversity was found in the therapies used by the respondents with 36 different AOX being listed (the top 10 AOX are listed in Table 5). It is in this context that there is a clear need for evidence-based guidelines for AOX treatment in male infertility.

3. Evidence based medicine

In the modern age, clinical care has shifted towards the practice of evidence-based medicine (EBM), defined as "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients" [55]. EBM incorporates the clinicians' experience and patient values with high quality medical research aiming to optimize clinical decision making. Amongst survey participants, AOX treatment was recommended mostly based on scientific evidence (69.1%) and personal experience (59.3%) (Table 6), reflecting some adherence to EBM guidelines.

Most of the clinical evidence investigating the ef-

ficacy of AOX therapy on male fertility comes from three published reviews by the Cochrane library in 2011, 2014, and 2019 [20,56,57]. The last update included 61 randomized clinical trials (RCTs) and collectively reported that AOX therapy for the treatment of male infertility is associated with a significant increase in pregnancy and live birth rates [20]. However, the quality of the reported evidence was low, which was attributed to the small study size and extensive heterogeneity across the included studies, with multiple AOX regimens, varied doses and different treatment durations. Moreover, the primary treatment groups were different. Some AOX trials were using it to boost natural or intrauterine insemination (IUI) conception, where others were aimed at augmenting *in vitro* fertilization-intracytoplasmic sperm injection (IVF-ICSI) outcomes in male factor infertility. As the latter is more reliant on improving sperm DNA quality, rather than motility or count, it is understandable that AOX therapy may be more advantageous in an IVF setting than in natural conception. Finally, many trials did not screen for OS and hence may include subjects who may not have OS and therefore would not benefit from AOX therapy.

Recently Agarwal et al [27] published clinical guidelines on the utility of AOX in the treatment of male infertility that were based on a systematic review and critical analysis of the evidence. The authors included 97 articles in their systematic review that were evaluated using multiple quality scores. They found that the majority of low-quality studies reported a significant improvement in semen parameters (85.7%) and sperm function tests (89.6%) following AOX therapy. Furthermore, 78.6% of low-quality studies showed a positive effect for AOX treatment on reproductive outcomes. While a significant effect was not detected among high-quality studies investigating the impact of AOX therapy on male fertility potential, 65% of low-quality studies investigating semen parameters, 58.3% sperm function and 60% reproductive outcomes, showed a positive effect. Overall, positive effects in semen parameters and sperm function following AOX therapy were reported by both the low- and high-quality studies for men with varicocele, abnormal semen parameters, IMI and UMI. This is supported by proteomic evidence that AOX therapy enhances AOX defence mechanisms at the subcellular level and is associated with overexpression of sperm proteins that are essential for the fertilization process [58].

Despite the reported data, recent practice statements published by international societies have showcased more negative views towards the use of AOX as a treatment modality for infertile men. The European Association of Urology (EAU) states that "No clear recommendation can be made for treatment of patients with idiopathic infertility using antioxidants, although antioxidant use may improve semen parameters (weak strength rating)" [59]. The American Society for Reproductive Medicine (ASRM) recommended that "clinicians should counsel patients that the benefits of supplements (*e.g.*, antioxidants, vitamins) are of questionable clinical utility in treating male infertility. Existing data are inadequate to provide recommendation for specific agents to use for this purpose. (Conditional Recommendation; Evidence Level: Grade B)" [60]. However, the extensive research that has been published on the study of AOX certainly warrants the formulation of new specific guidelines on the topic that would consider the utility of OS diagnostic testing, AOX type, dose and duration, and the clinical indications for AOX therapy.

4. Limitations of online survey

It is important to highlight some sources of bias which might have affected the collection of results through the online survey [61]. The survey link was shared around the world through the personal and professional society contacts of the experts associated with this study. The official and unofficial channels used might have created a selection bias, leading to an over- or under- representation of some countries in the results. Secondly, participants routinely prescribing AOX may have been more likely to fill out the survey, leading to participation bias [62]. There is also the impossibility to calculate a response rate, as it was not possible to keep track of the number of individuals who were given the opportunity to participate in the survey [63]. The length of the survey as well as the extensive number of questions might also have discouraged participants from completing it, leading to incomplete results.

Several strategies were put in place to reduce the bias of the results. First, the survey was carefully created, with several rounds of revisions by experts in the field. The aim was to collect reliable observations, by reducing the possibility of ambiguous, uncertain data

or misinterpretation [64]. The questionnaire was anonymous, to reduce the risk of desirability bias, where participants provide the feedback which is hypothetically-preferred [65]. The English language was also revised to make questions clearer and easily understandable for non-English speaking participants or for participants who spoke English as a second language. Furthermore, the online nature of the survey made it possible to reach a larger audience. Hence, it is reasonable to speculate that our results may represent a reliable and accurate global picture of the use of AOX in clinical practice for male infertility treatment.

5. Experts' opinion based on AOX survey results

This survey reports the opinions of 1,327 healthcare practitioners from around the world regarding the utility of AOX therapy for male infertility in clinical practice. Despite the recently published recommendations from international societies, including ASRM and EAU [60], 85.4% of respondents believe that AOX are a useful treatment option for men with infertility, with 69.1% of them basing their decision on scientific evidence in support for AOX use (Table 6). Indeed, such evidence exists, although the studies supporting the use of AOX are small and heterogeneous. As such, the question remains whether the quality of evidence is sufficient to justify the use of AOX therapy for male infertility, mainly when considering that it has a very low risk of side effects. High-quality evidence is difficult to obtain practically and is not currently available. The possibility of conducting placebo-controlled RCTs of AOX therapy is limited by the fact that patients can buy AOX over the counter at the local pharmacy or supermarket. As such, we may never be in a position to gather Level 1 evidence. In the meantime, clinical practice recommendations based on the currently available best evidence seems the most reasonable approach. Infertility is a unique medical condition which, despite the tremendous progress in its management, still suffers from many unknowns. Conception results from the highly complex interplay between a spermatozoon and an oocyte and is influenced by numerous variables, many of which are yet to be discovered. This is the reason why infertility is still unexplained in up to 30% of couples [50]. Furthermore, male factors causing an impairment in semen quality are unknown in about 50% of cases, and hence the term IMI [6]. AOX are dietary supplements that generally have a good safety profile and are supported by low-quality evidence showing that a positive effect on semen quality and reproductive function can be expected following treatment. Moreover, recent evidence revealed that when AOX are used for specific clinical indications (IMI and UMI), the improvement in semen quality has been shown by a high quality of evidence [27]. For these reasons, we advocate the use of AOX in clinical practice, provided that the following criteria are met:

1) Laboratory evidence of seminal OS. Testing for OS is recommended to identify the appropriate candidates for AOX therapy.
2) Consider treatment for a duration of 3–6 months, which is a sufficient intervention period to obtain a measurable impact on spermatogenesis.
3) Avoid excessive use of AOX, in high doses or for a prolonged duration, to avoid iatrogenic infertility due to reductive stress [33].
4) Finally, there is no consensus on the choice of AOX to be used. Based on physiological considerations, it would seem appropriate to use a combination of AOX that act on different physiological processes of the spermatozoa, such as preservation of energy metabolism, and improvement of sperm maturation and function, as well as providing protection from ROS.
5) AOX therapy should not be used to replace any other kind of treatment but should instead be seen as a viable synergistic option.

CONCLUSIONS

While the scientific evidence is of low quality, use of AOX in the management of infertile men is a common practice. Health care providers differ in their approach to AOX therapy, including evaluation of the OS status prior to commencing the treatment, the clinical indications for treatment, the AOX dose, duration, formulation, the end-point to be analysed, the follow up period, etc. Results from the present survey once again highlight the necessity for clinical practice guidelines on the use of AOX therapy in the management of the infertile male.

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14. Egyptian Society of Andrology (Taymour Mostafa, MD, Egypt; Mohamed Arafa, MD, Qatar)
15. Egyptian Foundation for Reproductive Medicine and Embryology (Medhat Amer, MD, Egypt)
16. ESHRE Certification for Reproductive Endoscopic Surgery (Hassan N. Sallam, MD, PhD, Egypt)
17. European Society for Gynaecological Endoscopy (Hassan N. Sallam, MD, PhD, Egypt)
18. Société d'andrologie de langue française (Edouard Amar, MD, France)
19. GIERAF (Groupe interAfricain d'étude, de recherche et d'application sur la fertilité) (Hassan N. Sallam, MD, PhD, Egypt)
20. Hong Kong Medical Association (Hyun Jun Park, MD, South Korea)
21. International Society of IVF (Hassan N. Sallam, MD, PhD, Egypt)
22. International Society of Sexual Medicine (Rafael Ambar, MD, Brazil)
23. Iranian Society for Reproductive Medicine (Mohammad Ali Sadighi Gilani, MD, Iran)
24. Iranian Urological Association (Mohammad Ali Sadighi Gilani, MD, Iran; Mohammad-Reza Moein, MD, Iran)
25. Japan Society of Assisted Reproduction (Yoshiharu Morimoto, MD, Japan)
26. Lebanese Urology Society (Ahmad Majzoub, MD, Qatar)
27. Mediterranean Society for Reproductive Medicine (Hassan N. Sallam, MD, PhD, Egypt)
28. Mexican Association for Reproductive Medicine (Israel Maldonado, PhD, Mexico)
29. Middle East Fertility Society (Hassan N. Sallam, MD, PhD, Egypt)
30. Moroccan Fertility Society (Hassan N. Sallam, MD, PhD, Egypt)
31. Red Latino Americana de Reproducción Asistida (Rafael F. Ambar, MD, Brazil)
32. Royal College of Obstetricians and Gynaecologists in Kuwait (Hassan N. Sallam, MD, PhD, Egypt)
33. Saudi Endometriosis Group (Hassan N. Sallam, MD, PhD, Egypt)
34. Saudi Fertility Group (Hassan N. Sallam, MD, PhD, Egypt)
35. Saudi Society for Obstetrics and Gynecology (Hassan N. Sallam, MD, PhD, Egypt)
36. Sociedad Argentina de Andrología (Rafael Ambar, MD, Brazil)
37. Société de la Médecine de la Reproduction (Edouard Amar, MD, France)
38. Societa' Italiana di Riproduzione Umana (Giovanni M. Colpi, MD, Italy)
39. Spanish Society of Andrology (Juan Alvarez, MD, Spain)
40. Togolese Fertility Society (Hassan N. Sallam, MD, PhD, Egypt)
41. Turkish Urological Society (Giovanni M. Colpi, MD, Italy)
42. Urological Society of Australia and New Zealand (USANZ) (Hyun Jun Park, MD, South Korea)
43. World Congress of IVF 2021 (Hassan N. Sallam, MD, PhD, Egypt)
44. World Journal of Men's Health (Hyun Jun Park, MD, South Korea)

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA. Data curation: MKPS, RF. Methodology: MKPS, RF. Project administration: AA, MKPS. Writing–original draft: AA, MKPS, RF, KL, DD, AM, RS, NT, NP, EK. Writing–review & editing: all authors.

Supplementary Material

Supplementary material can be found *via* https://doi.org/10.5534/wjmh.210025.

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