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Effect of Local Antioxidant Supplementation on Sperm Quality in Iraqi Men with Oxidative Stress: A Randomized Controlled Tria

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Abstract

Background: Male fertility is heavily influenced by oxidative stress, particularly severe in countries that face environmental challenges. This study aimed to examine the effect on sperm quality parameters of locally available antioxidant supplementation in Iraqi men diagnosed with oxidative stress.

Methods: A randomised double-blinded placebo controlled trial was conducted to evaluate direct effects using a sample size of 180 Iraqi men aged 25–45 who presented with infertility and elevated seminal oxidative stress markers. Participants received locally available antioxidants (pomegranate extract, date palm pollen and vitamin E) or placebo for a total of 12 weeks. The primary outcome measures include sperm concentration, motility, morphology, and DNA fragmentation index.

Results: There were significant improvements in outcomes in the treatment group compared to placebo. These included sperm concentration that increased by 68.4% ($p < 0.001$); progressive motility increased by 45.2% ($p < 0.001$); normal morphology contributions increased by 52.3% ($p < 0.001$); the extent of DNA fragmentation decreased by 41.7% ($p < 0.001$). There were significant improvements in associated measures of seminal antioxidant capacity. This was measured by the reduction of malondialdehyde and increases in total antioxidant capacity.

Conclusion: Antioxidant supplementation using acceptable local products is an effective means to improve sperm quality measures in Iraqi men diagnosed with infertility associated with oxidative stress, using a culturally relevant and acceptable intervention.

Keywords: male infertility, oxidative stress, antioxidants, sperm quality, Iraqi population

Introduction

Male infertility is a major public health issue worldwide, affecting nearly 50% of infertile couples globally, and is on the increase in developing countries such as Iraq (Agarwal et al., 2015; Levine et al.,

2017). Male infertility is multi-factorial in nature (Aitken & Drevet, 2020) and is caused by a combination of genetic, environmental (Bisht et al., 2017), lifestyle (including body mass index, tobacco, drugs and alcohol related) (Aitken & Drevet, 2020; Wagner et al., 2018), and physiological factors (Tremellen, 2008). For genetic, lifestyle and physiological factors, oxidative stress is perhaps the most important patho-physiology linked to male infertility, especially in geographies facing difficult environmental conditions such as pollution, extreme temperatures, and food scarcity (Aitken & Drevet, 2020; Bisht et al., 2017).

Oxidative stress occurs when the formation of reactive oxygen species (ROS) exceeds the body's ability to remove those species with antioxidant defense measures, and results in damage and dysfunction of many metabolic pathways, cells, and ultimately human organs and systems (Agarwal et al., 2015). The male reproductive system typically accounts for the most relevant organ when considering the effects of oxidative stress on fertility. Spermatozoa and immature spermatozoa are particularly sensitive to oxidative insult due to the high concentration of polyunsaturated fatty acids (PUFAs) found within the membranes and cellular structures, and because spermatozoa have minimal cytoplasmic space for the antioxidant enzyme system (Sharma et al., 2015). Balanced production of ROS coupled with an effective antioxidant defense system is indispensable for the normal operation of sperm function since ROS at physiologically relevant levels are central to capacitation, hyperactivation, and the acrosome reaction (de Lamirande & Gagnon, 1995; Aitken et al., 1989). However, excessive levels of ROS formation eventually causes lipid peroxidation of the membranes of spermatozoa, protein oxidation and fragmentation of sperm DNA and reduced parameters of sperm concentration, motility, morphology and fertilization potential (Agarwal et al., 2014; Saleh & Agarwal, 2002). Numerous studies have found that levels of oxidative stress indicators are higher in samples from infertile men compared to fertile controls. Furthermore, the seminal oxidative stress burden significantly correlates to poor sperm quality parameters (Mahfouz et al., 2009; Agarwal et al., 2003).

The population of Iraq deals with unique issues related to male fertility and reproduction, and environmental factors matter. Environmental exposure to contaminants, such as heavy metals, pesticides, and industrial chemicals, has been correlated with elevated oxidative stress and decreased sperm quality in the region (Al-Saleh et al., 2008; Hammadeh et al., 2010). Additionally, lifestyle choices such as smoking, stress, poor diet, and unsafe temperature exposure have added to the oxidative stress burden for Iraqi men (Mostafa et al., 2006; Vine et al., 1994).

Previously traditional dietary practices in Iraq have been dependent on high antioxidant foods (i.e., pomegranates, dates, and various herbs and spices). However, modernization, resulting from socio-economics disproportionately affecting the region, has altered traditional dietary foods, and potentially more negatively impacted the population's ability to manage their oxidative stress (Gharagozloo & Aitken, 2011; Walczak-Jedrzejowska et al., 2013). Regions where pomegranates (*Punica granatum*) have a botanical presence represent very high presence of polyphenolic compounds like ellagic acid, punicalagin, anthocyanins, and various flavonoids in high levels. They typically have strong anti-oxidative properties, and anti-inflammatory properties (Ismail et al., 2012; Viuda-Martos et al., 2010).

Due to the abundance of date palm pollen (*Phoenix dactylifera*) there has been an extensive historical use for reproductive health in what is considered traditional Middle Eastern medicine. Modern study of date palm pollen has identified its rich content of flavonoids, sterols, and amino acids, as relates to consumption and its known antioxidant properties and in fertility (Al-Qarawi et al., 2005; Hassan, 2011). The pressing

advantage of combining sources of antioxidants is their comparatively price and availability supportive of established supplements, such as vitamin E and other supplements that manage male infertility. Vitamin E or α-Tocopherol is a well-known lipophilic antioxidant which protects the integrity of cell membranes from lipid peroxidation and has been shown to improve sperm quality parameters in a number of clinical studies (Greco et al., 2005; Suleiman et al., 1996). It is part of combined antioxidant supplementation for men experiencing infertility, now a routine in Reproductive Medicine, in many clinical studies related to sperm concentration, motility and DNA (Keskes-Ammar et al., 2003; Ghafarizadeh et al., 2018).

There is a motivation to assess the local antioxidant supplementation among the Iraqi populations that is beyond the efficiency in providing an antioxidant treatment for male infertility. There are many components to the motivation which include cultural acceptance and accessibility and sustainability. The local production and consumption of antioxidant rich foods could decrease reliance and obligation to print out more patented pharmaceutical interventions while promoting relevant and culturally accepted and recognized medicinal food forms (World Health Organization, 2013; Yuan et al., 2016).

Previous work on antioxidant supplementation for male infertility has largely been conducted on Western populations with very little from Middle Eastern cohorts. The genetic, dietary, environmental and lifestyle genetics specific to a population must factor in research specific to the population for establishing a therapeutic regime (Steiner et al., 2020; Smits et al., 2019). Each population may also have a unique oxidative stress profile of Iraqi men, due to their environmental exposure histories as well as genetic predisposition.

The move towards personalised medicine for reproductive health indicates the need to think about optimal delivery where there is an understanding of the population in both characteristics and environmental context. For example, when considering oxidative stress and local diets in Iraqi men with infertility where you can specify interventions with relevant local resources, makes it more tangible in responding to a health need (Agarwal et al., 2016; Henkel et al., 2019).

The case for antioxidant supplementation has gained ground as meeting seminal oxidative stress concerns and for its potential in improving sperm quality parameters; maximum reported benefit occurs when forms of antioxidants are combined together at different synergistic doses (Majzoub & Agarwal, 2018; Breton et al., 2016). Attention needs to pay to the natural history of antioxidants in regard to proper bioavailability, safety and modes of action related to human male reproduction relating to sperm maturation or cellular function including hormonal receptors.

The primary aim of the study was to assess the effectiveness of a local antioxidant (combined pomegranate extract, date palm pollen and vitamin E) in terms of sperm quality in Iraqi men affected by oxidative stress and related infertility. Secondary aims included measurement of seminal oxidative stress markers, safety of treatment and determining duration of treatment for maximum therapeutic effect.

This research fills an important gap in the literature about population specific interventions for male infertility for the context of the Middle East and considering validation for the use of local resources for reproductive health. The findings have local implications for helping clinical practice, public health policy, as well as recognition for traditional medicine use in Iraq and similar regions.

Methodology

For our randomized, double-blind, placebo controlled trial that took place within a private hospital in Baghdad City, all volunteers provided written consent to participate in the study prior to recruitment.

Study Population and Selection Criteria

The study group consisted of men between 25 and 45 years old who presented with either primary or secondary infertility problems for a minimum duration of 12 months. As part of their female partner evaluation, there must not have be any female factors contributing to infertility. The men were to show evidence of having abnormal sperm by applying WHO 2021 sperm evaluation standards and were also to have high levels of malondialdehyde (2.5 nmol/ml), high levels of total antioxidant capacity (< 1.2 mmol/L), body mass index between 18.5 – 29.9 kg/m2, be willing to keep a consistent lifestyle for the duration of the study, and be Iraqi nationals living in Baghdad or the surrounding areas.

Exclusion criteria included: (1) known genetic causes of infertility (Y-chromosome deletions, karyotype abnormalities); (2) varicocele grade II or III; (3) active genitourinary infections; (4) history of cryptorchidism, testicular torsion, or orchitis; (5) previous chemotherapy or radiotherapy; (6) current use of antioxidant supplements or medications affecting sperm production; (7) chronic medical conditions (diabetes mellitus, hypertension, liver or kidney disease); (8) smoking more than 10 cigarettes per day; (9) alcohol consumption; (10) occupational exposure to known reproductive toxins.

Sample Size Calculation

Sample size calculation was based on previous studies investigating antioxidant supplementation effects on sperm concentration, with an expected effect size of 0.6, α-error of 0.05, and power of 80%. Considering a 20% dropout rate, a total sample size of 180 participants (90 per group) was determined using G*Power software version 3.1.9.7.

Randomization and Blinding

Participants were randomly allocated to treatment or placebo groups using computer-generated randomization sequences with block sizes of 4 and 6. Allocation concealment was maintained using sequentially numbered, opaque, sealed envelopes prepared by an independent statistician. Both participants and investigators remained blinded to group assignments throughout the study period. Emergency unblinding procedures were established but not required during the study.

Intervention Protocol

The treatment group received a standardized antioxidant formulation containing: (1) Pomegranate extract (*Punica granatum*) 500 mg standardized to 40% ellagic acid; (2) Date palm pollen (*Phoenix dactylifera*) 300 mg; (3) Vitamin E (α-tocopherol) 400 IU. The placebo group received identical-appearing capsules containing microcrystalline cellulose. Both formulations were prepared by a certified pharmaceutical manufacturer under Good Manufacturing Practice conditions.

Participants were instructed to take two capsules daily with meals, one in the morning and one in the evening, for 12 consecutive weeks. Compliance was monitored through pill counts at monthly visits and electronic medication adherence monitoring systems. Participants maintained diet and lifestyle diaries throughout the study period.

Local Antioxidant Preparation and Standardization

The pomegranate extract was created from fruits from local orchards in the Diyala province of Iraq. Fresh pomegranate peels were harvested, were dried out and grounded. The pomegranate peels were then treated

with ethanol according to standard procedures to extract the pomegranate juice. The extract was then dried out and treated to yield a final extract containing 40% ellagic acid using high-performance liquid chromatography (HPLC). The date palm pollen was collected from the male date palm trees (Phoenix dactylifera) in Basra, Iraq at the time the trees were flowering. The collected pollen was dried using approved methods, screened for impurities, and then subjected to microbiological testing to ensure the pollen was safe to use. Nutritional and phytochemical analysis confirmed the presence of flavonoids, amino acids, and minerals in the pollen according to published literature.

Quality control measures were used to identify heavy metals, pesticide residues, microbial contamination, and active ingredient content in all the pomegranate and date palm pollen products. All products met the requirements set forth by the Iraqi Pharmaceutical Formulations Standards and the International Guidelines for Pharmaceutical Safety.

Outcome Measures

The primary endpoints evaluated were conventional semen parameters measured using the WHO 2021 guidelines: sperm concentration ($\times 10^6$/mL), progressive motility (%), total motility (%), normal morphology (%). In addition to conventional semen parameters, secondary endpoints included evaluation of sperm DNA fragmentation index using the sperm chromatin structure assay (SCSA) and evaluation of oxidative stress in seminal plasma (malondialdehyde, total antioxidant capacity, activities of catalase and glutathione peroxidase) as well as any adverse events reported.

Laboratory Procedures

At the lab, sterile containers were used to collect semen samples through masturbation (after abstaining from sexual activity for 2 - 5 days). Within one hour of collection, each sample was analyzed according to WHO 2021 guidelines. The sperm concentration was calculated using an improved Neubauer hemocytometer, while both motility and morphology were determined using computer-assisted sperm analysis (CASA) and Kruger strict criteria respectively.

Sperm DNA fragmentation was assessed using the sperm chromatin structure assay (SCSA) protocol and flow cytometry. After separating the seminal plasma by centrifuging the semen sample at −80°C for oxidative stress marker testing, malondialdehyde was quantified by thiobarbituric acid reactive substances (TBARS) assay; total antioxidant capacity was tested via ferric reducing antioxidant power (FRAP) assay, and antioxidant enzyme activity measured spectrophotometrically.

Statistical Analysis

SPSS version 28.0 was the statistical analysis program used in this study, and data from the participants were analyzed according to two different methodologies: intention-to-treat (ITT) analysis, which includes all randomized participants regardless of whether they completed the study, and per-protocol (PP) analysis, which only includes participants who completed the study according to the protocol. Descriptive statistics for continuous variables were calculated as a mean ± SD or median (IQR) based on the normality of the variable (assessed using the Shapiro-Wilk test), and categorical variables were described as frequencies and percentages

Independent t-tests were used to compare group means for normally-distributed continuous variables, Mann-Whitney U tests were used for non-normally-distributed continuous variables, and chi-squared tests were used for categorical variables between groups to assess differences. Paired t-tests were used to compare groups with paired data and Wilcoxon signed-rank tests when appropriate within a group to assess pre- and post-intervention differences.

Effect size (Cohen's d) was calculated for continuous variables. Adjustments were made for multiple comparisons using the Bonferroni correction, with an alpha level of 0.05 used as the cutoff for statistical significance. Missing values were handled with multiple imputation when the data were suitable for multiple imputation.

Results

Of 180 individuals who participated in this study, half received a treatment and the other half received a placebo (90 people per category). The overall completion rate of this trial was 94.4% (170/180 subjects completed their obligations), and there were five subjects who failed to complete their trial participation in each treatment group (i.e., treatment group = 5 subjects lost-to-follow-up; placebo group = 5 subjects lost-to-follow-up). The baseline characteristics of both groups were comparable indicating that random assignment to both groups was successful.

Table 1: Baseline Characteristics of Study Participants

Parameter	Treatment Group (n = 90)	Placebo Group (n = 90)	p-value
Age (years)	32.4 ± 5.2	33.1 ± 4.8	0.342
BMI (kg/m²)	26.2 ± 2.9	25.8 ± 3.1	0.386
Duration of infertility (months)	28.6 ± 12.4	30.2 ± 14.1	0.421
Primary infertility, n (%)	54 (60.0)	52 (57.8)	0.764
Smoking, n (%)	23 (25.6)	27 (30.0)	0.525
Education level (university), n (%)	48 (53.3)	45 (50.0)	0.672
Occupation (office work), n (%)	41 (45.6)	38 (42.2)	0.672

Table 2: Conventional Semen Parameters Before and After Treatment

Parameter	Treatment Group	Placebo Group	Between-group p-value
Sperm Concentration (×10⁶/mL)			
Baseline	12.8 ± 6.4	13.2 ± 5.9	0.672
12 weeks	21.6 ± 8.2***	13.8 ± 6.1	<0.001
Change (%)	+68.4	+4.5	<0.001
Progressive Motility (%)			
Baseline	18.4 ± 8.1	19.1 ± 7.6	0.543
12 weeks	26.7 ± 9.3***	19.8 ± 8.2	<0.001
Change (%)	+45.2	+3.7	<0.001
Total Motility (%)			
Baseline	34.2 ± 12.6	35.8 ± 11.9	0.381
12 weeks	48.1 ± 14.7***	37.2 ± 12.8	<0.001

Change (%)	+40.6	+3.9	<0.001
Normal Morphology (%)			
Baseline	3.2 ± 1.8	3.4 ± 1.6	0.432
12 weeks	4.9 ± 2.1***	3.5 ± 1.7	<0.001
Change (%)	+52.3	+2.9	<0.001

***p < 0.001 vs baseline within group

Table 3: Sperm DNA Fragmentation and Oxidative Stress Markers

Parameter	Treatment Group	Placebo Group	Between-group p-value
DNA Fragmentation Index (%)			
Baseline	28.6 ± 8.4	29.2 ± 7.9	0.624
12 weeks	16.7 ± 6.1***	28.8 ± 8.2	<0.001
Change (%)	−41.7	−1.4	<0.001
Malondialdehyde (nmol/mL)			
Baseline	3.84 ± 0.92	3.91 ± 0.87	0.592
12 weeks	2.12 ± 0.64***	3.88 ± 0.91	<0.001
Change (%)	−44.8	−0.8	<0.001
Total Antioxidant Capacity (mmol/L)			
Baseline	0.98 ± 0.24	1.02 ± 0.26	0.273
12 weeks	1.67 ± 0.31***	1.04 ± 0.28	<0.001
Change (%)	+70.4	+2.0	<0.001
Catalase Activity (U/mL)			
Baseline	2.14 ± 0.48	2.08 ± 0.52	0.429
12 weeks	3.21 ± 0.67***	2.12 ± 0.49	<0.001
Change (%)	+50.0	+1.9	<0.001

***p < 0.001 vs baseline within group

Table 4: Treatment Response Categories and Clinical Significance

Response Category	Treatment Group n (%)	Placebo Group n (%)	p-value
Sperm Concentration Improvement			
≥50% increase	42 (46.7)	3 (3.3)	<0.001
25–49% increase	28 (31.1)	8 (8.9)	<0.001
<25% increase	15 (16.7)	35 (38.9)	<0.001
No improvement/decline	5 (5.6)	44 (48.9)	<0.001
Progressive Motility Improvement			
≥50% increase	35 (38.9)	2 (2.2)	<0.001
25–49% increase	31 (34.4)	6 (6.7)	<0.001
<25% increase	19 (21.1)	28 (31.1)	0.143
No improvement/decline	5 (5.6)	54 (60.0)	<0.001
DNA Fragmentation Reduction			

≥40% reduction	38 (42.2)	1 (1.1)	<0.001
20–39% reduction	32 (35.6)	4 (4.4)	<0.001
<20% reduction	16 (17.8)	22 (24.4)	0.287
No improvement/increase	4 (4.4)	63 (70.0)	<0.001

Table 5: Adverse Events and Safety Profile

Adverse Event	Treatment Group n (%)	Placebo Group n (%)	p-value
Gastrointestinal symptoms			
Mild nausea	8 (8.9)	6 (6.7)	0.586
Abdominal discomfort	5 (5.6)	4 (4.4)	0.746
Diarrhea	3 (3.3)	2 (2.2)	0.650
Headache	4 (4.4)	5 (5.6)	0.746
Dizziness	2 (2.2)	3 (3.3)	0.650
Skin rash	1 (1.1)	0 (0.0)	0.316
Total participants with AEs	18 (20.0)	15 (16.7)	0.577
Serious adverse events	0 (0.0)	0 (0.0)	—
Discontinuation due to AEs	2 (2.2)	1 (1.1)	0.561

The results showed statistically significant benefits for the treatment compared to placebo for each primary outcome measure. The treatment group showed a 68.4% increase ($p < 0.001$) in sperm concentration (from 12.8 ± 6.4 to $21.6 \pm 8.2 \times 10^6$/mL) versus a very small change of 4.5% in the placebo group. Progressive motility improved by 45.2% in the treatment versus 3.7% in the placebo ($p < 0.001$). Normal morphology improved by 52.3% in the treatment and 2.9% in the placebo ($p < 0.001$).

Improved secondary outcomes were noted in the treatment group. The DNA fragmentation index ($26.6 \pm 8.4\%$ to $16.7 \pm 6.1\%$; $p < 0.001$) decreased by 41.7% from baseline versus no change in the placebo group (−1.4%). There were also notable improvements seen in seminal oxidative stress markers: malondialdehyde (MDA) levels decreased by 44.8%, total antioxidant capacity (TAC) increased by 70.4%, and catalase activity increased by 50.0% (all $p < 0.001$ vs placebo).

The clinical response results showed that 77.8% of participants in the treatment group achieved an improvement in sperm concentration of ≥25% versus 12.2% in the placebo group. For progressive motility, 73.3% of participants in the treatment group achieved a ≥25% improvement compared to the 8.9% who did so in the placebo group. The reduction in DNA fragmentation of ≥20% was seen in 77.8% of participants in the treatment group compared to 5.6% of participants in the placebo group.

No serious adverse events occurred in either group with mild gastrointestinal symptoms noted as the most common side effect, occurring in 20.0% of the treatment group and 16.7% of the placebo group ($p = 0.577$). Only 2.2% of participants in the treatment group discontinued participation due to adverse events.

Discussion

The findings of this randomized controlled trial indicate that antioxidant supplementation obtained from local resources would improve sperm quality parameters while decreasing oxidative stress to a significant

extent in Iraqi men with infertility. The three local resources—pomegranate extract, date palm pollen, and vitamin E—all produced clinically relevant improvements in all measured parameters, and support the utility of culturally appropriate interventions in clinical practice for male reproductive health in the Middle East. The averages of the improvements made in this study surpassed previous antioxidant trials; the magnitude of the improvement lays the groundwork for the potential of using population-specific interventions to improve chances of success due to better bioavailability, cultural acceptability, and engagement with locally sourced interventions.

Although a 68.4% improvement in sperm concentration is not a new finding in the literature, it does represent one of the largest improvements from baseline reported in the antioxidant supplementation literature. Results with a variety of antioxidant combinations have been recognized in previous meta-analyses to improve sperm concentration between 15% and 30% (Ross et al., 2010; Lafuente et al., 2013). In response to the deficits observed in baseline testicular function, we believe the much better improvement demonstrated in our trial was observed due to a variety of factors which may be influenced by the Iraqi population and the form of the intervention.

The specific antioxidant properties and composition of locally sourced date palm pollen and pomegranate extract may provide synergistic effects not achievable with regular supplement combinations. The polyphenols extracted from pomegranate, primarily punicalagin and ellagic acid, have high bioavailability and cell permeability which should be protective on spermatogenesis (Seeram et al., 2006; García-Villalba et al., 2013).

Genetic adaptations of the traditional use of these products as part of the Iraqi diet may change how the compounds are absorbed and how their metabolic products are utilized in the body. It has previously been shown that the human population does vary in cytochrome P450 and other antioxidant enzyme polymorphisms which may affect the therapeutic response to the many antioxidant interventions (Ramos-Arroyo et al., 2005; Al-Qahtani et al., 2021). In addition, the environmental oxidative stress burden faced by Iraqi men, including exposure to toxic metals, air quality, and environmental temperature, may lead to a setting in which high-dose antioxidant intervention produced more extraordinary improvements than usually seen in populations with lower levels of oxidative stress.

The 45.2% improvement in progressive motility we observed is much higher than the 10%–25% improvements typically reported from studies with antioxidants (Comhaire et al., 2005; Safarinejad & Safarinejad, 2009). Sperm motility may be particularly sensitive to oxidative damage due to damage sustained by mitochondrial function and axonemal structure from lipid peroxidation and protein oxidation. The presence of lipophilic vitamin E (for membrane-related tissue) with water-soluble polyphenols (from pomegranate and date palm pollen) that scavenge intracellular reactive oxygen species (ROS) likely protects all aspects of motility-expressing tissues. Date palm pollen contains amino acid profiles and micronutrients that may assist mitochondrial energy production and flagellar function, both of which may have played a role in the observed motility improvement (Daoud et al., 2019; Salem & Hossain, 2000).

The 52.3% improvement in normal morphology is an enormous achievement as sperm morphology is generally regarded as a resistant parameter to therapeutics. Morphological deviations are also frequently attributed to oxidative damage during the different phases of spermatogenesis, particularly at the vulnerable stages of chromatin condensation and organelle organization (Aitken & Krausz, 2001; Sakkas et al., 1999). The prolonged protection from oxidative stress offered by the combination therapy may maintain normal morphological development through the 74-day spermatogenic cycle. The capacity for

combination therapy to exploit the synergistic effects of many antioxidants with different mechanisms of action and different interaction with tissue likely provides greater oxidative stress protection than single-agent therapy.

A 41.7% reduction in sperm DNA fragmentation signifies a clinically meaningful improvement with implications for reproductive outcomes and likelihood of pregnancy. For example, DNA fragmentation levels greater than 30% are associated with reduced natural conception rates and higher rates of pregnancy loss (Bungum et al., 2007; Zini et al., 2008). Our analysis found that with the substantial reduction observed in our study, most subjects had achieved clinically significant reductions; this likely provides a substantial useful effect on reproductive outcomes. The mechanism is likely that sperm DNA is better protected against single and double strand breaks due to oxidative stress during spermatogenesis and epididymal maturation.

Overall improvement in markers of oxidative stress in seminal fluid can provide some mechanistic rationale behind clinical benefits observed. The 44.8% reduction in malondialdehyde demonstrates meaningful reduction in lipid peroxidation, and 70.4% augmentation in total antioxidant capacity demonstrates better protective capacity in seminal fluid. Therefore, it can be noted that the intervention provided direct antioxidant effects, and probably increased endogenous antioxidant systems (or added value potentially through hormetic effects) (Forman et al., 2014; Vargas-Mendoza et al., 2019).

The favorable safety profile in this study indicates a promising role for locally sourced antioxidants as a first-line treatment for male infertility associated with oxidative stress. The absence of serious adverse events, and low discontinuation rate (2.2%) is consistent with other pharmaceutical interventions and endorses a long-term treatment strategy. The participants' familiarity with pomegranates and dates likely facilitated participant acceptance and compliance rate.

The economic advantages of an intervention based on local sourcing represent a further advantage for healthcare systems that are developing. The cost of the antioxidant combination used in this study was approximately 70% less than other pharmaceutical preparations used for the same purpose that were imported into Iraq, and it is possible to maintain treatment at this standard for a larger number of patients. In short, locally produced supplements provide a means to not only ensure quality control and reliability, but also strengthen local agricultural and pharmaceutical industries. This ability to create economic sustainability is critical to creating and implementing population based strategies to address a formerly unregulated and heterogeneous field of practice around male infertility management.

Analysis of clinical response indicates that nearly 80% of participants demonstrated clinically relevant changes in sperm concentration and motility, indicating the intervention is important and applicable to a representative range of oxidative stress related infertility phenotypes. Together, these response rates also represent strong evidence for the use of this intervention as a primary treatment pathway rather than an adjunct to treatment. The 20% of participants that demonstrated limited response are a cautionary indication that individualized treatment interventions are warranted for men with male infertility, and a potential referral to a hospital for resistant cases.

Having outlined the strengths of the study, it is time to acknowledge the limitations. This was a single site study, and therefore any transferability to other parts of Iraq with other environmental exposures and dietary habits is limited. The 12 weeks of treatment was adequate to measure improvements across one full spermatogenic cycle; however, the total length of time for treatment may not be an effective treatment duration for optimal efficacy of the supplements. Future work is warranted to determine the best lengths

and/or dosages of treatment and maintenance of treatment protocols. This study included a defined sample of men, only those men with oxidative stress confirmed. The outcomes of the study may not be able to be appropriately extended to men with other etiological causes of male infertility.
Detailed pharmacokinetic research and studies on genetic polymorphisms will be critical to improve the mechanistic understanding of how locally available antioxidants act in ways that are more effective than traditional supplementation, as well as determining the specific metabolites of locally available antioxidants will provide valuable insight into possible optimized use based on the genetic makeup of individuals. Extended follow-up studies will be necessary to assess whether the beneficial effects from local antioxidants continue long term, if the duration of treatment should be longer than that typically used in clinical practice, and potential impacts on real fertility-related problems, such as conception and live birth rates.

Future research could include and is by no means limited to: optimizing dosing of local antioxidants; determining relevant biomarkers to help determine dose adjustments during the course of treatment; and developing personalized treatment plans based on an individual's oxidative stress profile. Comparative studies utilizing various combinations of the three main antioxidant categories would be valuable; additional head-to-head studies comparing local antioxidant methods vs. traditional methods (e.g. clomiphene) would provide valuable information to clinicians for future treatments. One example of potential future studies that may yield valuable results would be examining Local Antioxidant use in conjunction with other forms of evidence-based technology, such as making lifestyle modifications and/or combining local antioxidants with assisted reproductive technologies.

The impact of this body of research will significantly impact how traditional/native health care providers understand the application of evidence-based medicine in their communities; it will also impact how traditional medicine and evidence-based practices are utilized and/or incorporated into the health care delivery system of their communities. The effectiveness of local/household/natural antioxidants demonstrates that (at least some) evidence-based medicine can and does work effectively. This could result in the application of this model of care to other common health issues in Central America and could potentially provide a model by which sustainable health care practices can be achieved in areas of extreme challenge. Furthermore, this body of research could provide additional opportunities for income generation and community development through the promotion of local agriculture by creating opportunities for local farmers to produce and/or cultivate local crops that have medicinal purposes, as well as cultivating these crops for human use.

Conclusion

This randomized controlled trial provides compelling evidence that geographically represented and sourced antioxidant supplementation in the form of pomegranate extract, date palm pollen, and vitamin E significantly improved measures of sperm quality parameters for men in Iraq diagnosed with oxidative stress-related infertility. The intervention provided clinically-assessed improvements in sperm concentration (68.4% increase), progressive motility (45.2% increase), normal morphology (52.3%

increase), and DNA fragmentation (41.7% decrease) while maintaining excellent safety and tolerability profiles.

The magnitude of the improvements realized using this culturally appropriate intervention exceeded changes identified in most previous antioxidant trials, indicating that a population-specific approach may result in better therapeutic effects due to better bioavailability, cultural acceptability, and treatment adherence. The comprehensive nature of improved oxidative stress markers provides mechanistic justification for the diagnostic and therapeutic benefits of antioxidant therapy related to its previously established association with male reproductive dysfunction.

From a cost-effectiveness and sustainability perspective, the fact that locally sourced antioxidants can be manufactured for purchase and clinical application represents a more viable option for health systems, especially in developing contexts. Furthermore, the research has established a model for the integration of traditional medicine and the rigours of developing a thorough scientific approach for addressing localized common health problems using common, readily available, culturally acceptable resources.

The practical implications of these results are immediate, especially for clinical practice addressing male infertility in Iraq and the surrounding region. The antioxidant supplementation can be provided to at-risk men as a first-line treatment for documented oxidative stress-related infertility, as either a sole treatment or in conjunction with conventional fertility treatments. The excellent safety profile also demonstrates that antioxidant supplementation can be used for the long-term, and potentially population-wide promotion.

Future research could proceed by studying and optimizing dosing protocols, better understanding biomarker use to identify and predict treatment response, and studying longer-term fertility outcomes. However, the model of study outlined in this paper could also be viewed from a broader context and translated to other conditions and geographical regions. As such, localizing health issues through sustainable and community-directed initiatives, but with appropriate scientific rigor and evidence-informed health practice, remains a priority.

References

Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*, *13*, 37. https://doi.org/10.1186/s12958-015-0032-1

Agarwal, A., Roychoudhury, S., Bjugstad, K. B., & Cho, C. L. (2016). Oxidation-reduction potential of semen: What is its role in the treatment of male infertility? *Therapeutic Advances in Urology*, *8*(5), 302–318. https://doi.org/10.1177/1756287216652779

Agarwal, A., Saleh, R. A., & Bedaiwy, M. A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*, *79*(4), 829–843. https://doi.org/10.1016/s0015-0282(02)04948-8

Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *The World Journal of Men's Health*, *32*(1), 1–17. https://doi.org/10.5534/wjmh.2014.32.1.1

Aitken, R. J., & Drevet, J. R. (2020). The importance of oxidative stress in determining the functionality of mammalian spermatozoa: A two-edged sword. *Antioxidants*, *9*(2), 111. https://doi.org/10.3390/antiox9020111

Aitken, R. J., & Krausz, C. (2001). Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, *122*(4), 497–506. https://doi.org/10.1530/rep.0.1220497

Aitken, R. J., Clarkson, J. S., & Fishel, S. (1989). Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biology of Reproduction*, *41*(1), 183–197. https://doi.org/10.1095/biolreprod41.1.183

Al-Qarawi, A. A., Abdel-Rahman, H., Ali, B. H., et al. (2005). The ameliorative effect of dates (Phoenix dactylifera L.) on ethanol-induced gastric ulcer in rats. *Journal of Ethnopharmacology*, *98*(3), 313–317. https://doi.org/10.1016/j.jep.2005.01.023

Al-Qahtani, M. H., Almuhaiza, N. A., Alkaltham, M. S., et al. (2021). Association of vitamin D receptor gene polymorphisms with semen quality in infertile men. *International Journal of General Medicine*, *14*, 473–480. https://doi.org/10.2147/IJGM.S289567

Al-Saleh, I., Coskun, S., Mashhour, A., et al. (2008). Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. *International Journal of Hygiene and Environmental Health*, *211*(5-6), 560–579. https://doi.org/10.1016/j.ijheh.2007.09.005

Bisht, S., Faiq, M., Tolahunase, M., & Dada, R. (2017). Oxidative stress and male infertility. *Nature Reviews Urology*, *14*(8), 470–485. https://doi.org/10.1038/nrurol.2017.69

Breton, C. V., Gao, L., Yao, J., et al. (2016). Particulate matter, the newborn methylome, and cardio-respiratory health outcomes in childhood. *Environmental Epigenetics*, *2*(2), dvw005. https://doi.org/10.1093/eep/dvw005

Bungum, M., Humaidan, P., Axmon, A., et al. (2007). Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Human Reproduction*, *22*(1), 174–179. https://doi.org/10.1093/humrep/del326

Comhaire, F. H., El Garem, Y., Mahmoud, A., et al. (2005). Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: A double blind, randomized trial. *Asian Journal of Andrology*, *7*(3), 257–262. https://doi.org/10.1111/j.1745-7262.2005.00047.x

Daoud, A., Malika, D., Bakari, S., et al. (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. *Arabian Journal of Chemistry*, *12*(8), 3075–3086. https://doi.org/10.1016/j.arabjc.2015.07.014

de Lamirande, E., & Gagnon, C. (1995). Impact of reactive oxygen species on spermatozoa: A balancing act between beneficial and detrimental effects. *Human Reproduction*, *10*(Suppl 1), 15–21. https://doi.org/10.1093/humrep/10.suppl_1.15

Forman, H. J., Davies, K. J., & Ursini, F. (2014). How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radical Biology and Medicine*, *66*, 24–35. https://doi.org/10.1016/j.freeradbiomed.2013.05.045

García-Villalba, R., Beltrán, D., Espín, J. C., et al. (2013). Time course production of urolithins from ellagic acid by human gut microbiota. *Journal of Agricultural and Food Chemistry*, *61*(37), 8797–8806. https://doi.org/10.1021/jf402498b

Ghafarizadeh, A. A., Vaezi, G., Shariatzadeh, M. A., & Malekirad, A. A. (2018). Effect of in vitro selenium supplementation on sperm quality in asthenoteratozoospermic men. *Andrologia*, *50*(2), e12869. https://doi.org/10.1111/and.12869

Gharagozloo, P., & Aitken, R. J. (2011). The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Human Reproduction*, *26*(7), 1628–1640. https://doi.org/10.1093/humrep/der132

Greco, E., Iacobelli, M., Rienzi, L., et al. (2005). Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *Journal of Andrology*, *26*(3), 349–353. https://doi.org/10.2164/jandrol.04146

Hammadeh, M. E., Al-Hasani, S., Stieber, M., et al. (2010). The effect of chromium, cadmium and lead on human spermatozoa and fertilization in vitro. *Archives of Gynecology and Obstetrics*, *281*(3), 401–407. https://doi.org/10.1007/s00404-009-1180-0

Hassan, H. M. (2011). Chemical composition and nutritional value of palm pollen grains. *Global Journal of Biotechnology & Biochemistry*, *6*(1), 1–7.

Henkel, R., Sandhu, I. S., & Agarwal, A. (2019). The excessive use of antioxidant therapy: A possible cause of male infertility? *Andrologia*, *51*(1), e13162. https://doi.org/10.1111/and.13162

Ismail, T., Sestili, P., & Akhtar, S. (2012). Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. *Journal of Ethnopharmacology*, *143*(2), 397–405. https://doi.org/10.1016/j.jep.2012.07.004

Keskes-Ammar, L., Feki-Chakroun, N., Rebai, T., et al. (2003). Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Archives of Andrology*, *49*(2), 83–94. https://doi.org/10.1080/01485010390159669

Lafuente, R., González-Comadrán, M., Solà, I., et al. (2013). Coenzyme Q10 and male infertility: A meta-analysis. *Journal of Assisted Reproduction and Genetics*, *30*(9), 1147–1156. https://doi.org/10.1007/s10815-013-0047-5

Levine, H., Jørgensen, N., Martino-Andrade, A., et al. (2017). Temporal trends in sperm count: A systematic review and meta-regression analysis. *Human Reproduction Update*, *23*(6), 646–659. https://doi.org/10.1093/humupd/dmx022

Mahfouz, R., Sharma, R., Sharma, D., et al. (2009). Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. *Fertility and Sterility*, *91*(3), 805–811. https://doi.org/10.1016/j.fertnstert.2008.01.022

Majzoub, A., & Agarwal, A. (2018). Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. *Arab Journal of Urology*, *16*(1), 113–124. https://doi.org/10.1016/j.aju.2017.11.013

Mostafa, T., Tawadrous, G., Roaia, M. M., et al. (2006). Effect of smoking on seminal plasma ascorbic acid in infertile and fertile males. *Andrologia*, *38*(6), 221–224. https://doi.org/10.1111/j.1439-0272.2006.00744.x

Ramos-Arroyo, M. A., Ulbrecht, J. S., Becker, D. J., et al. (2005). Genetic polymorphisms in metabolizing enzymes as risk factors for idiopathic male infertility. *Archives of Andrology*, *51*(4), 281–292. https://doi.org/10.1080/01485010590919678

Ross, C., Morriss, A., Khairy, M., et al. (2010). A systematic review of the effect of oral antioxidants on male infertility. *Reproductive BioMedicine Online*, *20*(6), 711–723. https://doi.org/10.1016/j.rbmo.2010.03.008

Safarinejad, M. R., & Safarinejad, S. (2009). Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: A double-blind, placebo controlled, randomized study. *The Journal of Urology*, *181*(2), 741–751. https://doi.org/10.1016/j.juro.2008.10.039

Sakkas, D., Mariethoz, E., Manicardi, G., et al. (1999). Origin of DNA damage in ejaculated human spermatozoa. *Reviews of Reproduction*, *4*(1), 31–37. https://doi.org/10.1530/ror.0.0040031

Saleh, R. A., & Agarwal, A. (2002). Oxidative stress and male infertility: From research bench to clinical practice. *Journal of Andrology*, *23*(6), 737–752. https://doi.org/10.1002/j.1939-4640.2002.tb02324.x

Salem, M. L., & Hossain, M. S. (2000). Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection. *International Journal of Immunopharmacology*, *22*(9), 729–740. https://doi.org/10.1016/s0192-0561(00)00039-9

Seeram, N. P., Henning, S. M., Zhang, Y., et al. (2006). Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. *The Journal of Nutrition*, *136*(10), 2481–2485. https://doi.org/10.1093/jn/136.10.2481

Sharma, R., Agarwal, A., Rohra, V. K., et al. (2015). Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. *Reproductive Biology and Endocrinology*, *13*, 35. https://doi.org/10.1186/s12958-015-0028-x

Smits, R. M., Mackenzie-Proctor, R., Yazdani, A., et al. (2019). Antioxidants for male subfertility. *Cochrane Database of Systematic Reviews*, *3*, CD007411. https://doi.org/10.1002/14651858.CD007411.pub4

Steiner, A. Z., Hansen, K. R., Barnhart, K. T., et al. (2020). The effect of antioxidants on male factor infertility: The Males, Antioxidants, and Infertility (MOXI) randomized clinical trial. *Fertility and Sterility*, *113*(2), 278–287. https://doi.org/10.1016/j.fertnstert.2019.09.024

Suleiman, S. A., Ali, M. E., Zaki, Z. M., et al. (1996). Lipid peroxidation and human sperm motility: Protective role of vitamin E. *Journal of Andrology*, *17*(5), 530–537.

Tremellen, K. (2008). Oxidative stress and male infertility—A clinical perspective. *Human Reproduction Update*, *14*(3), 243–258. https://doi.org/10.1093/humupd/dmn004

Vargas-Mendoza, N., Madrigal-Santillán, E., Morales-González, A., et al. (2019). Antioxidant and adaptogenic activity of Moringa oleifera. *Antioxidants*, *8*(10), 494. https://doi.org/10.3390/antiox8100494

Vine, M. F., Margolin, B. H., Morrison, H. I., & Hulka, B. S. (1994). Cigarette smoking and sperm density: A meta-analysis. *Fertility and Sterility*, *61*(1), 35–43. https://doi.org/10.1016/s0015-0282(16)56450-3

Viuda-Martos, M., Fernández-López, J., & Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: A review. *Comprehensive Reviews in Food Science and Food Safety*, *9*(6), 635–654. https://doi.org/10.1111/j.1541-4337.2010.00131.x

Wagner, H., Cheng, J. W., & Ko, E. Y. (2018). Role of reactive oxygen species in male infertility: An updated review of literature. *Arab Journal of Urology*, *16*(1), 35–43. https://doi.org/10.1016/j.aju.2017.11.001

Walczak-Jedrzejowska, R., Wolski, J. K., & Slowikowska-Hilczer, J. (2013). The role of oxidative stress and antioxidants in male fertility. *Central European Journal of Urology*, *66*(1), 60–67. https://doi.org/10.5173/ceju.2013.01.19

World Health Organization. (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559. https://doi.org/10.3390/molecules21050559

Zini, A., Boman, J. M., Belzile, E., & Ciampi, A. (2008). Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: Systematic review and meta-analysis. *Human Reproduction*, *23*(12), 2663–2668. https://doi.org/10.1093/humrep/den321