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Article

Involvement of Oxidative Stress in Decreased Number and Motility of Sperm in Men without Obvious Causes for Male Infertility

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Abstract: Background: Male factors contribute to approximately 50% of infertile couples. However, obvious causes remain unknown in many cases. This observational study aimed to investigate the associations of clinical and lifestyle parameters with sperm parameters. **Materials and Methods:** This study enrolled 41 men of infertile couples without obvious causes for male infertility from July 2023 to April 2024. Semen samples were evaluated for sperm number, motility, DNA fragmentation, and oxidative stress (OS) marker oxidation-reduction potential (ORP). Blood samples were analyzed for biochemical parameters, including advanced glycation end products (AGEs) and systemic OS marker diacron-reactive oxygen metabolites (d-ROMs). Skin-accumulated AGEs levels were identified with an autofluorescence method. Lifestyle factors were assessed with a lifestyle questionnaire. **Results:** Most of the participants were under 40 years old and non-obese with normal clinical and sperm parameters. Multiple regression analyses revealed that body mass index, serum d-ROMs, and semen ORP levels were independently associated with decreased sperm number. Additionally, serum zinc and semen ORP levels were associated with sperm motility. Furthermore, serum zinc and high-density lipoprotein-cholesterol levels were associated with sperm progressive motility and DNA fragmentation, respectively. The rest of the clinical and lifestyle factors, including skin-accumulated and serum AGE levels were not correlated with any sperm parameters. Furthermore, serum d-ROMs and semen ORP levels were not correlated with each other or any of the clinical and lifestyle factors. **Conclusions:** Our present study indicates that both systemic and local OS may be independently involved in sperm abnormality of healthy men without obvious causes for male infertility.

Keywords: Advanced glycation end products; Male infertility; Oxidative stress; Spermatozoa

1. Introduction

The infertility rate has been increasing in many countries, and approximately one in every six individuals at reproductive ages is estimated to experience infertility in their lifetime [1,2]. Reportedly, male infertility is implicated in approximately half of the cases of couples unable to conceive [1,2]. Various biological and environmental factors can cause male infertility, but no obvious causes of infertility are reported to be observed in 30%–50% of male patients [1,2]. Obesity and undesirable lifestyles, such as smoking habits and alcohol overconsumption, are suspected to be risk factors for male infertility, but the underlying mechanism for this association remains unclear [3–5].

Accumulating evidence has indicated that obesity and undesirable lifestyle facilitate advanced glycation end products (AGEs) formation and oxidative stress (OS) generation, both of which play pathogenic roles in the development and progression of various types of chronic non-communicable diseases, including atherosclerotic cardiovascular disease, diabetes, and chronic kidney disease [6–8]. AGEs are molecules formed through macromolecule nonenzymatic glycation, such as proteins, lipids, and nucleic acids [6–8]. The formation and accumulation have progressed under hyperglycemic and/or chronic inflammatory conditions, and diet is a major environmental source of AGEs in the

human body [6–8]. AGEs have been shown to induce OS generation and inflammation reactions in many cell and tissue types through the interaction with its cell-surface receptor, a receptor for AGEs (RAGE) [6–8]. We have recently revealed that AGE-RAGE axis activation contributes to decreased total number, motility, and viability of sperm in a mouse model of diabetes with obesity partly by inducing testicular OS and inflammation [9]. Furthermore, many clinical studies have revealed that OS can impair the male reproduction system [10–15]. These results indicate that AGEs and OS may be one of the markers related to obesity and undesirable lifestyle habits to male infertility without obvious causes. However, the association of AGEs and OS with sperm abnormality in apparently healthy men who lack obvious causes for male infertility remains unclear.

Therefore, we conducted an observational clinical study enrolling men of couples with infertility who lacked obvious causes for male infertility. In the present study, we utilized serum diacron-reactive oxygen metabolites (d-ROMs) and sperm oxidation-reduction potential (ORP) levels as systemic and local OS markers, respectively, and evaluated their correlations with various sperm parameters, such as total number, total and progressive motility, and DNA fragmentation of sperm. Here, we further examined the association of skin accumulation and circulating AGE levels with decreased total number and impaired function of sperm, independent of OS.

2. Materials and Methods

2.1. Ethics Statement

This observational study was conducted at Showa University Hospital from July 2023 to April 2024. The Ethics Committee of Showa University reviewed and approved the present study protocol (Approval No: 2023-047-B, approval date: July 18, 2023).

2.2. Study Participants

This study enrolled men of couples with infertility who visited the Reproduction section of the Obstetrics and Gynecology Department at Showa University Hospital (Tokyo, Japan). The exclusion criteria were (1) men who were <20 years old; (2) those with a current or past history of pyospermia/azoospermia, testicular injury or infection, varicocele, vasal reconstruction, or excessive exposure to radiation/chemical; (3) those with active malignant or inflammatory disease; (4) those with chronic disease such as chronic lung or liver failure; and (5) those who were deemed inappropriate for inclusion as evaluated by their respective physicians.

2.3. Study Design

All participants were evaluated with routine clinical and physical examination, lifestyle habits questionnaire [16], semen and blood sample collection in non-fasting conditions, and skin-accumulated AGE measurement. The questionnaire comprised 12 multiple-choice questions: (1) exercise frequency, (2) smoking habit duration, (3) alcohol consumption frequency, (4) sleep duration, (5) mental stress degree, (6) vegetable consumption, (7) breakfast frequency, (8) overeating habit, (9) greasy food consumption, (10) processed food consumption, (11) sugary food consumption, and (12) vegetable-first eating habit [16]. Each question was scored from 1 (the worst) to 5 (the best), and the sum of the scores was utilized as an individual value. All procedures were performed following the ethical standards of Showa University on human experimentation and the Helsinki Declaration of 1964 and its later version. Informed consent was obtained from all subjects involved in the study.

2.4. Laboratory Measurements

Systemic OS levels were evaluated by measuring serum hydroperoxide levels with the d-ROMs test (Wismerll Company Limited, Bunkyo, Tokyo, Japan) as previously described [17]. The d-ROMs test results were expressed in Caratelli Units (U.CARR) [17]. Serum AGE levels were identified with an enzyme-linked immunosorbent assay [18]. Serum glucose, lipids, zinc (Zn), and free testosterone

levels were measured with an enzyme electrode method, colorimetric assay, atomic absorption spectrophotometry, and radioimmunoassay, respectively. Skin-accumulated AGE levels were measured on the dorsal side of the forearms with a non-invasive autofluorescence method with AGE-Reader™ Mu (Diagnoptics Technologies B.V., Groningen, Netherlands), as previously reported [16].

2.5. Sperm Parameter Measurement

Semen samples were collected by masturbation after 2–7 days of sexual abstinence and analyzed following the World Health Organization (WHO) laboratory manual for the examination and processing of human semen, sixth edition [19]. The total number, motility, and progressive motility of sperm were measured under a light microscope. A single-blinded investigator (L.C.) conducted all microscopic measurements to avoid technical bias.

2.6. Semen Oxidative Stress Measurement

Local OS levels were evaluated by measuring the ORP of semen samples with the MiOXSYS™ analyzer (Aytu BioScience, Englewood, CO, USA). In brief, liquefied semen of 30 µL was loaded into the disposable test sensor, which was pre-inserted into the MiOXSYS analyzer, which measures electron transfer from reductants (antioxidants) to oxidants, reflecting the overall balance between oxidants and antioxidants. Higher static ORP levels indicate oxidative stress due to an imbalance between oxidant and antioxidant activity.

Semen ORP levels were then normalized by dividing the measured ORP values by sperm concentration (10⁶/mL) [11,12].

2.7. Sperm DNA Fragmentation Measurement

Sperm DNA fragmentation was evaluated using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method as previously reported with some modifications [20]. Sperm smears were incubated with dithiothreitol of 10 mM for 1 min and further with lithium diiodosalicylate of 10 mM and dithiothreitol of 1 mM for 2 h, fixed in 4% paraformaldehyde for 1 h, permeabilized with 2% Triton X-100 and 0.1% bovine serum albumin for 15 min, and incubated with the primary antibody (Product ID: 11684795910; Sigma-Aldrich Japan, Meguro, Tokyo, Japan) at 4°C for overnight. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Product ID: D1306; Thermo Fisher Scientific, Waltham, MA USA). Sperm DNA fragmentation was presented as a percentage of TUNEL-positive sperm to DAPI-positive sperm.

2.8. Statistical Analyses

JMP Pro statistical software version 17.0.0 (SAS Institution Inc., Cary, NC, USA) was used for statistical analyses. The normality of data distribution was tested with the Shapiro–Wilk test. Data with normal and non-normal distributions were expressed as mean ± standard deviation (SD) and median with 25 and 75 percentiles, respectively. Categorical data were presented as percentages. Simple and multiple stepwise regression analyses were conducted with dependent variables, including the total number, total motility percentage, progressive motility percentage, and DNA fragmentation percentage of sperm. Independent variables were age, body weight, waist, body mass index (BMI), serum d-ROMs, skin-accumulated AGEs, serum glucose, serum total cholesterol (TC), serum high-density lipoprotein-cholesterol (HDL-C), serum triglycerides (TG), serum Zn, serum free testosterone, serum AGEs, current smoking habit, current drinking habit, total lifestyle habits questionnaire score, abstinence period, and semen ORP. Two groups were compared with unpaired *t*-test, Wilcoxon signed-rank test, or Fisher's exact test, as appropriate. Statistical significance was set at *p*-values of <0.05.

3. Results

3.1. Background Clinical and Lifestyle Characteristics

The analyses in the present study included 41 participants. Table 1 presents the ages and medical histories of the study participants and their partners, and Table 2 shows the background clinical and lifestyle characteristics of the study participants. Most of the participants were <40 years old with their BMI <25.0 kg/m². Serum d-ROMs levels were close to the upper limit of the normal range, which is <300 U.CARR [21]. Skin-accumulated AGE levels were within the normal range after adjusting for ages [16]. Serum glucose, TC, HDL-C, TG, Zn, and free testosterone levels were within the normal range of non-fasting conditions. Of the study participants, <25% had current smoking habits, whereas >75% had current drinking habits.

Table 1. Ages and medical histories of the study participants and their partners.

Participants (men)	Values	Partners (women)	Values
Number	41	Number	41
Age (years old)	37.5 ± 6.3	Age (years old)	35.8 ± 4.6
Comorbid disease		Comorbid disease	
Hypertension, <i>n</i> (%)	2 (4.9)	Uterine fibroids, <i>n</i> (%)	10 (24.4)
Type 2 diabetes, <i>n</i> (%)	2 (4.9)	Ovulation dysfunction, <i>n</i> (%)	6 (14.6)
Hyperuricemia, <i>n</i> (%)	1 (2.4)	Endometriotic cyst, <i>n</i> (%)	3 (7.3)
Hypercholesterolemia, <i>n</i> (%)	1 (2.4)	Sexual dysfunction, <i>n</i> (%)	2 (4.9)
Depression, <i>n</i> (%)	1 (2.4)	Polycystic ovary syndrome, <i>n</i> (%)	1 (2.4)
		Uterus duplex, <i>n</i> (%)	1 (2.4)
		Garves' disease, <i>n</i> (%)	1 (2.4)
		Hypothyroidism, <i>n</i> (%)	1 (2.4)
		Rheumatoid arthritis, <i>n</i> (%)	1 (2.4)

Data are expressed as mean ± standard deviation (SD).

Table 2. Background clinical and lifestyle characteristics of study participants.

Parameters	Values
Body weight (kg)	72.5 (65.6–82.4)
BMI (kg/m ²)	24.5 (22.5–26.3)
Waist (cm)	86.7 (80.0–94.1)
Serum d-ROMs (U.CARR)	299.0 (276.0–333.5)
Skin AGEs (AF)	1.7 ± 0.3
Serum glucose (mg/mL)	111.5 ± 24.4
Serum TC (mg/dL)	96.5 ± 13.4
Serum HDL-C (mg/dL)	47.5 (39.4–59.1)
Serum TG (mg/dL)	119.2 ± 60.9
Serum Zn (µg/dL)	83.5 ± 18.7
Serum-free testosterone (pg/mL)	12.2 (10.3–15.1)
Serum AGEs (µg/dL)	0.1 ± 0.1
Current smoking habit (%)	22.0
Current drinking habit (%)	75.6
Lifestyle habits questionnaire score	36.8 (32.5–43.0)

Data are expressed as mean ± standard deviation (SD), median with 25 and 75 percentiles, and percentage for data with normal distribution, non-normal distribution, and category, respectively.

BMI: body mass index, d-ROMs: diacron-reactive oxygen metabolites, AGEs: advanced glycation end products, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol,

TG: triglycerides. Reference values at non-fasting conditions: glucose of <200 mg/dL; TC of <220 mg/dL; HDL-C of >40 mg/dL; TG of <150 mg/dL; Zn of 80–130 µg/dL; free testosterone of >11.8pg/mL. The questionnaire comprised 12 multiple-choice questions that were scored from 1 (the worst) to 5 (the best). The sum of the questions was utilized as an individual value, and the most and least healthy lifestyle corresponds to 60 and 12, respectively.

3.2. Background Sperm Parameters

Table 3 shows the sperm parameters of the study participants. According to the WHO laboratory manual for the examination and processing of human semen, sixth edition [19], all the participants exhibited semen volume, sperm concentration, and total sperm number within the normal ranges. However, total and progressive motility of sperm were impaired in some of the participants, accounting for 14.6% and 12.2% of participants with abnormal values, respectively. Additionally, semen ORP levels and sperm DNA fragmentation percentages of all the participants were below the reported cut-off values for male infertility [11,22].

Table 3. Background sperm parameters of study participants.

Parameters	Values
Abstinence period (days)	5.1 ± 4.0
Semen volume (mL)	3.3 ± 1.1
Sperm concentration (10 ⁶ /mL)	157.8 ± 100.7
Total sperm number (10 ⁶ /ejaculate)	527.8 ± 396.0
Sperm motility (%)	62.2 (49.0–73.7)
Progressive motility of sperm (%)	56.5 (40.4–66.8)
Semen ORP level (mV/10 ⁶ sperm/mL)	0.3 ± 0.3
Sperm DNA fragmentation (%)	7.6 ± 7.8

Data are expressed as mean ± SD, median with 25 and 75 percentiles, or percentage, as appropriate. Normal values for sperm parameters according to reference #19: semen volume of >1.4 mL; sperm concentration of >16 × 10⁶/mL; sperm number of >390 × 10⁶/ejaculate; abnormal motility of <42%; abnormal progressive motility of <30%. Reference values for semen ORP and sperm DNA fragmentation: semen ORP of <1.34 mV/10⁶ sperm/mL [11]; sperm DNA fragmentation of <19.25% [22]. ORP: oxidation-reduction potential.

3.3. Simple and Multiple Regression Analyses of Clinical and Lifestyle Variables related to Sperm Parameters

Table 4 presents simple regression analyses of clinical and lifestyle variables related to sperm parameters. Of the significant variables, body weight (inversely), BMI (inversely), serum d-ROMs levels (inversely), abstinence period, and semen ORP levels (inversely) were correlated with total sperm number; serum Zn and semen ORP levels (inversely) with total motility of sperm; serum Zn level with progressive motility of sperm; and body weight (inversely), BMI (inversely), waist (inversely), and serum HDL-C level with sperm DNA fragmentation.

Table 5 demonstrates the multiple stepwise regression analyses with the same variables, excluding body weight and waist because body weight, BMI, and waist were closely correlated with each other. Hence, BMI was selected for the analyses to avoid multicollinearity. BMI, serum d-ROMs level, and semen ORP level were inversely associated with the total number of sperm. Serum Zn levels and semen ORP levels (inversely) were independent correlates of the total motility of sperm. Serum Zn and HDL-C levels were the sole independent correlates of the progressive motility of sperm and its DNA fragmentation, respectively.

Table 4. Simple regression analyses of clinical and lifestyle parameters related to sperm parameters.

Sperm parameters Variables	Total number		Total motility		Progressive motility		DNA fragmentation	
	β	p	β	p	β	p	β	p
Age (years old)		0.81		0.24		0.23		0.38
Body weight (kg)	-0.36 [-0.60 – -0.06]	0.02		0.55		0.65	-0.37 [-0.61 – -0.07]	0.02
BMI (kg/m ²)	-0.39 [-0.63 – -0.10]	0.01		0.35		0.40	-0.35 [-0.59 – -0.05]	0.02
Waist (cm)	-0.27 [-0.54 – -0.04]	0.08		0.50		0.56	-0.40 [-0.63 – -0.11]	<0.01
Serum d-ROMs (U.CARR)	-0.46 [-0.67 – -0.17]	<0.01		0.28		0.31		0.72
Skin AGEs (AF)		0.28		0.28		0.33		0.34
Serum glucose (mg/mL)		0.06		0.07		0.09		0.21
Serum TC (mg/dL)		0.32		0.74		0.64		0.73
Serum HDL-C (mg/dL)		0.85		0.54		0.69	0.40 [0.10 – 0.63]	0.01
Serum TG (mg/dL)		0.67		0.47		0.31		0.09
Serum Zn (µg/dL)		0.81	0.38 [0.08 – 0.62]	0.01	0.39 [0.09 – 0.62]	0.01		0.24
Serum-free testosterone (pg/mL)		0.80		0.76		0.92		0.15
Serum AGEs (µg/dL)		0.36		0.22		0.30		0.33
Current smoking habit (%)		0.94		0.81		0.56		0.93
Current drinking habit (%)		0.73		0.98		0.83		0.32
Lifestyle habits questionnaire score		0.58		0.27		0.20		0.68
Abstinence period (days)	0.37 [0.07 – 0.61]	0.02		0.90		0.76		0.19
Semen ORP (mV/10 ⁶ sperm/mL)	-0.47 [-0.68 – -0.19]	<0.01	-0.32 [-0.57 – -0.15]	0.04		0.07		0.57

β shows the regression coefficient with 95% confidential interval.

Table 5. Multiple stepwise regression analyses of clinical and lifestyle parameters related to sperm parameters.

Variables	Total number (<i>R</i> ² = 0.42, <i>p</i> < 0.01)		Total motility (<i>R</i> ² = 0.20, <i>p</i> = 0.01)		Progressive motility (<i>R</i> ² = 0.13, <i>p</i> = 0.01)		DNA fragmentation (<i>R</i> ² = 0.14, <i>p</i> = 0.01)	
	<i>β</i>	<i>p</i>	<i>β</i>	<i>p</i>	<i>β</i>	<i>p</i>	<i>β</i>	<i>p</i>
BMI (kg/m ²)	-0.29 [-0.54 – -0.04]	0.02						
Serum d-ROMs (U.CARR)	-0.42 [-0.66 – -0.17]	<0.01						
Serum Zn (μg/dL)			0.37 [0.09 – 0.66]	0.01	0.39 [0.09 – 0.62]	0.01		
Semen ORP (mV/10 ⁶ sperm/mL)	-0.34 [-0.59 – -0.09]	<0.01	-0.31 [-0.87 – -0.17]	0.03				
Serum HDL-C (mg/dL)							0.40 [0.10 – 0.63]	0.01

The following parameters were utilized as variables for the multiple stepwise regression analyses: Age, BMI, serum d-ROMs, skin AGEs, serum glucose, serum TC, serum HDL-C, serum TG, serum Zn, serum free testosterone, serum AGEs, current smoking habit, current drinking habit, total score of lifestyle habits questionnaire, abstinence period, and semen ORP. *R*² shows the adjusted coefficient of determination. *β* shows the regression coefficient with 95% confidential interval.

3.4. Comparison of Clinical, Lifestyle, and Sperm Parameters in Two Subgroups with Higher- vs. Lower-Serum d-ROMs Levels

Subgroup analysis, focusing on serum d-ROMs levels, was conducted. The participants were evenly categorized into two subgroups based on their serum d-ROMs levels. Table 6 presents the clinical, lifestyle, and sperm parameters of the subgroups. Serum glucose levels appeared greater in the higher-serum d-ROMs group, with no significant differences in other clinical and lifestyle parameters between the two groups. The lower-serum d-ROMs group exhibited greater semen volume, motile sperm concentration, total sperm number, total motile sperm number, and progressive motile sperm number compared with the higher-serum d-ROMs group. No significant difference was found in other sperm parameters between the subgroups.

Table 6. Metabolic and lifestyle parameters in two subgroups categorized based on baseline serum d-ROMs levels.

	Higher serum d-ROMs group	Lower serum d-ROMs group	<i>p</i>
Number	21	20	
Age (years old)	38.0 ± 5.8	37.0 ± 7.0	0.62
Body weight (kg)	73.4 (65.4–82.4)	72.1 (66.1–82.2)	0.72
BMI (kg/m ²)	24.7 (22.7–26.8)	24.1 (21.0–26.4)	0.47
Waist (cm)	88.8 (79.4–97.2)	85.3 (80.1–93.8)	0.54
Serum d-ROMs (U.CARR)	329 (310–367)	276 (248–283)	<0.01
Skin AGEs (AF)	1.7 ± 0.2	1.8 ± 0.3	0.40
Serum glucose (mg/mL)	118.6 ± 25.8	104.1 ± 20.9	0.06
Serum TC (mg/dL)	96.1 ± 13.2	96.9 ± 13.8	0.84
Serum HDL-C (mg/dL)	44.0 (39.4–58.6)	49.3 (40.8–62.3)	0.43
Serum TG (mg/dL)	120.5 ± 63.7	117.9 ± 59.4	0.89
Serum Zn (µg/dL)	85.8 ± 18.0	81.1 ± 19.6	0.43
Serum-free testosterone (pg/mL)	11.0 (10.0–14.3)	13.2 (10.8–16.5)	0.16
Serum AGEs (µg/dL)	0.2 ± 0.1	0.1 ± 0.1	0.88
Current smoking habit (%)	28.6	15.0	0.45
Current drinking habit (%)	76.2	75.0	1.00
Lifestyle habits questionnaire score	37.0 (34.0–43.0)	35.5 (29.0–43.5)	0.45
Abstinence period (days)	4.4 ± 4.0	5.9 ± 3.9	0.26
Semen volume (mL)	2.9 ± 0.8	3.7 ± 1.3	0.02
Sperm concentration (10 ⁶ /mL)	131.1 ± 87.5	185.8 ± 108.1	0.08
Total sperm number (10 ⁶ /ejaculate)	383.1 ± 264.2	679.7 ± 457.5	0.02
Sperm motility (%)	61.1 (44.1–71.6)	68.3 (49.9–77.9)	0.15
Sperm progressive motility (%)	54.2 (35.1–64.6)	58.6 (42.6–70.2)	0.11
Semen ORP (mV/10 ⁶ sperm/mL)	0.3 ± 0.2	0.3 ± 0.4	0.84
Sperm DNA fragmentation (%)	7.9 ± 7.9	7.3 ± 8.0	0.82

Data are presented as the mean ± SD, median with 25 and 75 percentiles, or percentage as appropriate.

3.5. Simple Regression Analyses of Clinical and Lifestyle Parameters Related to Serum d-ROMs and Semen ORP levels

Finally, we examined the associations of clinical, biochemical, and lifestyle parameters, including skin-accumulated and circulating AGE levels, with serum d-ROMs and semen ORP levels in simple regression analyses. None of the clinical, biochemical, or lifestyle parameters was significantly associated with serum d-ROMs or semen ORP levels (data are not shown). Furthermore, serum d-ROMs and semen ORP levels were not correlated with each other (*p* = 0.50).

4. Discussion

This study investigated the associations of clinical and lifestyle habits with sperm parameters in men of couples with infertility who lacked an obvious cause for male infertility. Most of the study participants were under 40 years old and non-obese with normal biochemical and sperm parameters. We revealed that serum and semen OS levels assessed by d-ROMs and ORP, respectively, besides BMI, were independently associated with the decreased total number of sperm. Furthermore, semen OS levels were correlated with reduced total motility of sperm. Serum Zn levels were positively associated with total and progressive motility of sperm among the other clinical and lifestyle parameters, whereas HDL-C levels were independently correlated with DNA fragmentation of sperm. The rest of the clinical and biochemical parameters and lifestyle habits, including skin-accumulated and serum AGE levels were not correlated with any sperm parameters. Additionally, we revealed that serum d-ROMs and semen ORP levels were not correlated with any of the clinical, biochemical, or lifestyle parameters. Furthermore no correlation between serum d-ROMs and semen ORP levels was found, which is consistent with previous studies [23,24]. These results indicate that semen OS levels are independently associated with decreased number and motility of sperm even in men with almost normal sperm parameters, whereas systemic OS was independently correlated with reduced sperm number. Sperm number and motility are crucial factors for male fertilization ability; thus, our present study indicates the involvement of OS in sperm abnormality of health men without obvious causes for male infertility.

Sperm are vulnerable to OS-induced damage because of their high polyunsaturated fatty acid contents, antioxidant enzyme deficiency, and DNA repair capacity limitation [10,13]. Semen ORP has been generally used as a sperm OS indicator [11,12], and the levels have been higher in men with abnormal sperm quality than in those with normal sperm quality [11]. Furthermore, the same study [11] revealed that, in men with at least one abnormal sperm parameter, semen ORP levels were inversely correlated with semen parameters, such as sperm concentration, total count, total and progressive motility, and normal morphological form, and that the cut-off value for abnormal sperm quality was calculated as 1.34 mV/10⁶ sperm/mL. On the otherhand, a recent study showed that semen ORP levels were not associated with sperm parameters in men with idiopathic male infertility [23]. However, the involvement of semen ORP in decreased total number and impaired total and progressive motility of sperm remains unknown in participants with almost normal sperm parameters. In this study, >85% of participants had normal number and motility and semen ORP levels were below the reported cut-off value, but semen OS levels were independently correlated with decreased total number and motility of sperm. This result indicates that, even below the reported cut-off value, semen ORP levels are inversely associated with sperm number and motility of healthy men.

Systemic OS has negatively affected fertilization ability in men, in addition to semen OS [14,15]. Plasma levels of malondialdehyde (MDA), a marker of lipid peroxidation [25], and leukocyte 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage were significantly higher in infertile men without obvious infertility causes compared with fertile men, both of which were correlated with each other [14]. Moreover, leukocyte 8-OHdG was inversely associated with sperm count and total and progressive motility of sperm, whereas plasma MDA was inversely associated with progressive motility of sperm [14]. Additionally, another study revealed higher plasma MDA and leukocyte OS levels, whereas plasma antioxidant capacity was lower in patients with idiopathic male infertility than in age-matched normozoospermic controls, and plasma MDA levels were inversely correlated with the total number and concentration of sperm in these patients [15]. The present study assessed systemic OS levels with the d-ROMs assay, which measures the total amount of hydroperoxides in serum [17,21]. We revealed in this study that serum d-ROMs levels were correlated with decreased total number of sperm, independent of semen ORP levels and other clinical parameters. The higher-serum d-ROMs group demonstrated lower semen volume and total, motile, and progressive motile sperm number compared to the lower-serum d-ROMs group, but no significant differences in clinical or biochemical parameters or semen ORP levels between the two subgroups. The median values in the higher- vs. lower-serum d-ROMs groups were 329 vs. 276

U.CARR and serum d-ROMs levels of <300, 300–320, and >320 U.CARR were considered to correspond to normal, borderline, and increased OS conditions, respectively [21]. Thus, our present results indicate that increased systemic OS may contribute to a decreased total number of sperm. Spermatogenesis is initiated from spermatogonia located in the basal membrane of seminiferous tubules, thereby being affected by systemic factors [26]. Conversely, spermatocytes produced from spermatogonia via mitosis are supported by the epithelium of Sertoli cells, which comprises blood-testis-barrier, and then differentiate into sperm, which cannot be affected by systemic factors [26]. Therefore, increased systemic OS assessed by higher d-ROMs levels may affect sperm production rather than sperm functional maturation through a mechanism distinct from local OS.

Previous studies have revealed that obesity negatively affects male fertilization ability [5]. The present study revealed that the mean BMI of the study participants was 24.5 kg/m², which falls within the non-obese range [27], but BMI was associated with decreased total number of sperm, independent of the other clinical or biochemical factors. Higher BMI may become a marker of visceral obesity that could be related to adipokine disturbance and chronic inflammation [28], thereby contributing to a decreased total number of sperm in concert with systemic OS. Further, this study revealed that the mean serum Zn levels of the study participants were within the normal range, but Zn levels were positively correlated with total and progressive motility of sperm, which was consistent with the previous result [29]. Optimal BMI control and Zn supplementation may be potential therapeutic targets for ameliorating the fertilization ability in men with infertility without obvious causes of infertility.

The present study has several limitations. First, clinical factors that contributed to increased systemic and semen OS in our participants were unknown. Second, the exact reason for the association of HDL-C levels with DNA fragmentation, which was not consistent with the previous observations, was unknown [30,31]. We evaluated nutrient intake with the questionnaire, but not with food records; thus, dietary factors may have confounded the present results. Third, we revealed no significant correlation between serum and skin-accumulated AGE levels and sperm parameters. Semen contains a large amount of fructose as a main energy source of sperm, the levels of which are approximately 300-fold higher than those of other body fluids [32]; thus, AGE measurement tools utilized in this study may not work for detecting fructose-derived AGEs. Finally, this is a cross-sectional study, and thus it cannot elucidate the causal relationship between systemic and semen OS and sperm abnormality.

6. Conclusions

Our present study indicates that both systemic and local OS may be independently involved in sperm abnormality of healthy men without obvious causes for male infertility.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

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