Original Article

The effects of tomato juice on male infertility

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Background and Objectives: This study aimed to investigate the effects of tomato juice consumption on seminal plasma lycopene levels and sperm parameters in infertile men. **Methods and Study Design:** Subjects were male infertility patients with poor sperm concentration (<20×10^6/mL) and/or motility (<50%). Following a four-week observation period, subjects were randomly assigned among three groups: a tomato juice group, an antioxidant group, and a control group. The subjects in the tomato juice group and the antioxidant group daily consumed one can of tomato juice (containing 30 mg of lycopene) or one antioxidant capsule (containing vitamin C 600 mg, vitamin E 200 mg, and glutathione 300 mg), respectively, for 12 weeks (feeding period). Seminal plasma lycopene levels and sperm parameters were measured every 6 weeks during the feeding period. **Results:** Forty-four patients completed the study (control group: 12, antioxidant group: 15, tomato juice group: 17). In the tomato juice group, plasma lycopene level was significantly increased at the 12th week of the feeding period. Moreover, a decrease in seminal plasma white blood cells and an increase in sperm motility in the tomato juice group were statistically significant at the 12th and 6th weeks, respectively, compared to the control group. In the antioxidant capsule group, no significant improvement was observed in semen parameters. **Conclusions:** In conclusion, regular consumption of tomato juice seems to improve sperm motility in infertile patients. This is the first report to show that commercially available food, such as tomato juice, might be beneficial for male infertility.

Key Words: tomato, lycopene, male infertility, oxidative stress, sperm parameters

INTRODUCTION

Worldwide, 13-15% of couples are infertile,[1] defined as the inability to achieve pregnancy within 12 months of regular sexual intercourse for couples. These infertile couples seek medical treatment to improve their chances of fertility and successful pregnancy. Male factors account for 25-50% of causes.[2,3] About 60% of male infertility may be due to a genetic factor, with environmental and host factors accounting for the rest. Many environmental and host factors are known, including industrial chemicals, hormonal imbalances, alcohol consumption, and smoking.[4] However, the reason these factors cause male infertility has not been fully clarified. A recent report identified oxidative stress as a likely cause.[5] Many environmental and host factors may cause oxidative stress through excessive generation of reactive oxygen species (ROS); these damage spermatozoa by oxidizing cell membranes, which contain large amounts of unsaturated fatty acids. Lipid peroxidation of spermatozoa leads to a loss of membrane integrity and an increase in permeability, inactivation of cellular enzymes, and cell apoptosis. The consequence is reduced sperm count and activity, decreased motility, and abnormal morphology.[6,7] Therefore, enhancement of antioxidant capacity to protect spermatozoa from oxidative stresses could present a major opportunity for improving male infertility.[8-10] Many clinical studies have been performed on the possible

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beneficial effects of treatment with antioxidants on male infertility.[5]

Lycopene is a red pigment found in fruits and vegetables, including tomatoes, watermelon, and apricots, and is reportedly one of the most efficient singlet oxygen quenchers and peroxyl radical scavengers.[11] Moreover, there are many reports that lycopene has a beneficial role in prevention of chronic diseases, such as cardiovascular disease, atherosclerosis, cancer, and neurodegenerative disorders.[12] Humans cannot synthesize lycopene, and must consume vegetables and fruits that contain it.[13] Lycopene absorbed from the intestine is carried by the bloodstream in lipoprotein particles, and is distributed to various tissues.

Lycopene is reportedly highly concentrated in the male testis.[14] Therefore, lycopene was expected to improve male infertility by enhancing antioxidant capacity of sperm, prompting a few clinical trials. Gupta reported that administration of lycopene (2 mg twice a day for 3 months) improved many semen parameters in men with idiopathic nonobstructive oligo/astheno/teratozoospermia.[15] However, this is insufficient to conclude that lycopene is beneficial for male infertility. Furthermore, there is no report evaluating the effect of consumption of foods containing lycopene. Accordingly, we conducted an intervention study to clarify the beneficial effects of tomato juice, which is rich in lycopene, on male fertility.

MATERIALS AND METHODS

Study design

We conducted a parallel group study with the approval of the Ethics Committees of the International University of Health and Welfare (IUHW) Hospital, and Kagome Co., Ltd. Following a four-week observation period, subjects were assigned by the Department of Pharmacy, IUHW Hospital, among three groups: a tomato juice group (n=21), an antioxidant group (n=17), and a control group (n=16). The subjects in the tomato juice group and the antioxidant group daily consumed one can of tomato juice or one capsule of an antioxidant pill, respectively, for 12 weeks (feeding period). The subjects in the control group were not administered any experimental foods, and were required to avoid lycopene-rich foods containing tomatoes through the experimental period. Semen samples were collected at 0, 6, and 12 weeks, and blood samples were drawn at 0 and 12 weeks of the feeding period.

Experimental foods

A commercially available tomato juice ("Natsushibori"; Kagome Co., Ltd., Japan) containing 30 mg of lycopene, 38 mg of vitamin C, and 3 mg of vitamin E in one can (190 g) was used as the experimental food for the tomato juice group. The capsules for the antioxidant group contained vitamin C (CINAL Combination Tablet 600 mg/day, Shionogi Pharmaceutical Co., Japan), vitamin E (Juvela N Soft Capsule 200 mg/day, Tanabe Seiyaku Hanbai Co., Japan), and glutathione (Tathion Tablet 300 mg/day, Eisai Co., Japan).

Subjects

We recruited male infertility patients with poor sperm concentration ($<20\times10^6$/mL) and/or motility (<50%) according to WHO criteria 1999.[16] The candidates were interviewed by a doctor, and those who smoked, had a tomato allergy, or had a history of relevant illness, such as adult-onset mumps orchitis, undescended testicles, or a high semen white blood cell (WBC) count ($\geq1\times10^6$/mL) were excluded. Consequently, 54 subjects aged 26-50 (average: 36.9) participated in the experiment.

Plasma samples

A morning blood sample was drawn from the antecubital vein for each patient, to reduce the effect of diurnal variation in hormone levels, and the serum was sent to the laboratory at the IUHW Hospital. Blood samples drawn into a test tube containing disodium ethylenediaminetetraacetic acid (EDTA) were centrifuged at 1,087 g for 20 minutes, and the plasma samples were stored at -80°C until levels of baseline characteristics (testosterone, follicle-stimulating hormone [FSH], luteinizing hormone [LH]),) and lycopene were quantified.

Baseline characteristics of plasma

Levels of testosterone were measured by an electrochemiluminescence immunoassay using Testosterone II (SRL, Japan). Levels of FSH and LH were determined using a time-resolved immunofluorometric assay (SRL, Japan). Measurements of these three hormones were carried out by SRL, Inc.

Semen samples and parameters

The participants provided semen samples by masturbation in a room close to the laboratory. In the laboratory, the samples were kept at 37°C until analyzed. The men had been asked to abstain from ejaculation for at least 48 hours prior to participation in the study. The actual abstinence period was calculated as the time between the current and previous ejaculation, based on self-reported information. Semen volume was assessed by aspirating the entire sample into a graduated 5 mL syringe (Terumo, Tokyo, Japan), after it was liquefied at 37°C. Sperm concentration, motility, and parameters of sperm movement (VCL = curvilinear velocity, μm/s; VSL = straight-line velocity, μm/s; ALH = amplitude of lateral head displacement, μm; and STR = straightness and sperm head pitch, μm) were evaluated by computer-assisted semen analysis (CASA) (Hamilton Thorne Ceros, USA), using a sperm motility analyzer system (SMAS) (Kashimura, Japan). For the assessment of sperm concentration, the samples were diluted in a FertiCult Flushing medium without insulin (FertiPro, Belgium). A 10 μm-deep Makler counting chamber (Oriental Instruments Co., Japan) was used for measurements of semen by CASA. Smears were prepared for morphological evaluation and stained (Diff-Quick; Sysmex, Japan), and 200 cells were finally assessed according to strict criteria,[17] yielding the total abnormal rate. The number of leukocytes was assessed using Bürker-Türk hemocytometers (Kayagakilri-kakogyo, Japan). The rest of the seminal plasma samples were stored at -80°C until the levels of lycopene and malondialdehyde (MDA) were quantified.

Plasma and seminal plasma lycopene

Plasma and seminal plasma lycopene concentrations were

determined by high performance liquid chromatography (HPLC). The plasma sample (200 μL) or seminal plasma sample (500 μL) was withdrawn, and 1.0 mL of an ethanol solution of trans-β-8'-apocarotenal was added to the plasma as an internal standard. A solution of *n*-hexane and dichloromethane (4:1, v/v, 5.0 mL) was added to the mixture and centrifuged (1,087 *g*, 10 minutes). The supernatant (4.0 mL) was evaporated under nitrogen gas. The residue was dissolved in 200 μL of solvent mixture (hexane/acetone/ethanol/toluene, 10:7:6:7, v/v/v/v), filtered with a 0.20 μm filter, and HPLC analysis was performed, using a photodiode array detector (SPD-M10, Shimadzu, Japan) at a detecting wavelength of 472 nm, and a C30 carotenoid column (250×4.6 mm, 5 μm; YMC, Wilmington, NC, USA).

Physical examination

Physical examination of the infertile men was performed by a urologist (TI). Testis disposition and varicoceles were evaluated with the infertile subject standing. For assessment, the same type of punched-out orchiometer was used.[18]

Seminal plasma MDA

MDA concentration was spectrophotometrically determined using OxiSelect TBARS assay kit (Cell Biolabs, San Diego, CA, USA).

Statistical analysis

Statistical analyses were performed using SPSS PC (version 5.0) software. Tukey's test for multiple comparisons was employed as a post-hoc ANOVA for lycopene and MDA levels compared to baseline. Dunnett's test for multiple comparisons was employed as a post-hoc ANOVA for comparing semen parameters between control and test substance groups. Semen parameters were normalized by cube root transformation before analysis to correct for the skewed distribution.

RESULTS

Table 1 shows baseline characteristics of the study subjects. These were similar among all three groups, with no significant difference. A total of 54 patients were recruited, but only 44 completed the entire study (12/16, control group; 15/17, antioxidant group; 17/21, tomato juice group). The reason for dropout was loss of follow-up in 8 (2 in the control group, 2 in the antioxidant group, and 4 in the tomato juice group), and successful fertilization with a partner in 2 (2 in the control group).

Figure 1 shows mean values and ranges of plasma and seminal plasma lycopene levels during the experimental period. Regular consumption of tomato juice for 12 weeks significantly increased the plasma lycopene level (p=0.017). The lycopene level in seminal plasma was about one two-hundredth of that in blood plasma. Seminal plasma lycopene levels were also increased at the 12th week in the tomato juice group (p=0.023). There was a significant correlation between blood plasma and seminal plasma lycopene level (r=0.36, p=0.024).

Figure 2 describes the mean values and ranges of seminal plasma MDA. Sample measurement was incomplete (control group: 4, antioxidant group: 8, tomato juice

Table 1. Baseline characteristics of study patients in the three groups

	Control group	Antioxidant group	Tomato juice group
Age (years)	36.2±1.91	36.1±0.86	38.1±1.76
Duration of infertility (years)	2.34±0.70	3.20±0.42	2.92±0.42
Rt. testis volume (mL)	19.5±1.01	19.4±1.18	20.0±1.02
Lt. testis volume (mL)	18.8±1.10	18.9±1.20	20.4±0.73
FSH (mIU/mL)	5.35±0.99	6.01±1.19	5.64±1.27
LH (mIU/mL)	2.75±0.33	2.56±0.22	2.82±0.49
Testosterone (ng/mL)	5.49±0.58	4.96±0.35	5.34±0.52

FSH: follicle-stimulating hormone; LH: luteinizing hormone.
Data are presented as mean ± standard error of the mean (SEM).

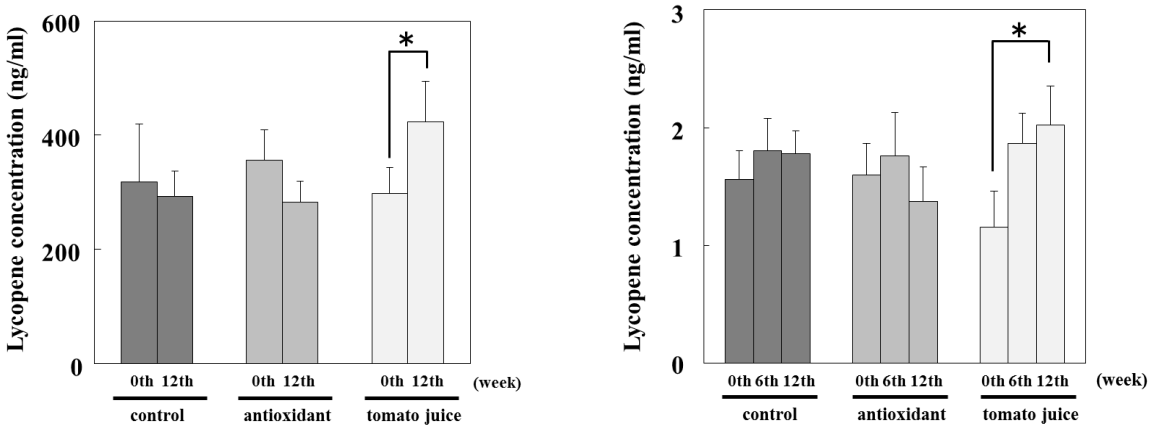

Figure 1. Mean lycopene levels in plasma (left) and seminal plasma (right) in the three groups. Data are presented as mean±SEM.
*p<0.05 vs 0th week

Table 2. Changes in semen parameters in the three groups

Semen parameter	Control group			Antioxidant group			Tomato juice group		
	0^{th} week	6^{th} week	12^{th} week	0^{th} week	6^{th} week	12^{th} week	0^{th} week	6^{th} week	12^{th} week
Semen volume (mL)	3.70±0.57	4.19±0.67	4.10±0.47	3.73±0.40	3.72±0.47	3.25±0.39	3.70±0.33	3.56±0.34	3.49±0.35
Sperm concentration ($\times 10^6$/mL)	51.5±7.62	65.5±22.5	38.4±8.87	55.6±9.46	61.0±13.5	43.6±6.72	49.4±7.30	65.3±10.8	50.0±8.34
Sperm motility (%)	31.2±3.33	27.7±4.66	30.3±6.35	28.6±3.12	26.3±4.37	33.8±4.97	29.5±2.94	35.4±3.69	31.2±3.47
Abnormal sperm rate (%)	77.4±1.59	78.3±2.30	81.6±1.39	83.8±1.41	81.1±2.30	81.4±2.03	80.7±1.40	81.5±1.59	81.7± 0.15
Semen plasma WBC ($\times 10^6$/mL)	0.68±0.12	0.73±0.18	0.65±0.19	0.65±0.14	0.68±0.26	0.62±0.10	0.65±0.09	0.63±0.17	0.44±0.06
Straight-line velocity (μm/s)	23.7±1.39	22.9±2.73	21.8±1.40	24.4±1.47	25.0±2.91	23.4±2.18	21.1±0.80	21.7±1.39	22.7±1.20
Survilinear velocity (μm/s)	55.8±4.25	56.4±5.73	54.0±4.18	58.1±3.40	61.0±5.13	55.3±5.23	51.5±2.03	54.2±3.07	54.6±2.54
Straightness (%)	44.1±1.25	38.3±2.98	42.0±2.26	42.0±2.17	41.1±2.27	40.4±3.47	42.2±1.23	40.1±1.02	42.6±1.28
Amplitude of lateral head displacement (Hz)	0.39±0.06	0.49±0.13	0.34±0.07	0.40±0.06	0.49±0.09	0.34±0.04	0.29±0.03	0.36±0.04	0.74±0.38
Sperm head pitch (μm)	10.1±0.59	9.11±0.82	12.2±1.12	10.4±0.23	10.6±0.54	9.55±0.74	10.9±0.61	10.0±0.46	10.1±0.53
Degree of sperm-nucleus damage (%)	27.2±3.60	37.5±5.83	31.3±5.00	20.3±2.21	23.0±3.10	21.2±4.00	27.2±2.49	27.5±3.03	26.9±3.22

Data are presented as mean±SEM.

Table 3. Changes from the beginning of the experimental period in the three groups

Semen parameter	Control group		Antioxidant group		Tomato juice group	
	6^{th} week	12^{th} week	6^{th} week	12^{th} week	6^{th} week	12^{th} week
Period of abstinence (days)	1.5±2.5	0.2±3.7	1.2±1.3	-1.1±0.7	-0.8±1.5	0.3±1.5
Semen volume (mL)	0.45±0.28	0.40±0.41	-0.01±0.20	-0.48±0.22*	-0.13±0.23	-0.21±0.20*
Sperm concentration ($\times 10^6$/mL)	12.8±20.6	-13.0±9.46	5.38±7.80	-11.9±5.48	15.9±7.29	0.64±6.76
Sperm motility (%)	-3.24±3.35	-0.87±4.77	-2.28±3.76	5.24±5.28	5.87±3.26*	0.08±2.93
Abnormal sperm rate (%)	0.89±1.36	4.21±2.13	-2.67±1.74	-2.41±1.46	0.82±1.77	1.56±1.46
Semen plasma WBC ($\times 10^6$/mL)	0.05±0.15	-0.02±0.16	0.02±0.17	-0.03±0.09	-0.01±0.18	-0.21±0.08*
Straight-line velocity (μm/s)	-0.67±2.51	-1.87±1.00	0.63±2.20	-1.02±1.59	0.65±1.36	1.86±1.28
Curvilinear velocity (μm/s)	0.54±4.72	-1.80±2.9	2.86±3.45	-2.84±3.42	2.67±2.74	3.27±2.66
Straightness (%)	-5.33±2.57	-2.16±2.26	-0.91±1.77	-1.66±2.41	-1.27±1.55	0.42±1.87
Amplitude of lateral head displacement (Hz)	0.09±0.12	-0.04±0.06	0.09±0.06	-0.06±0.04	0.06±0.05	0.42±0.37
Sperm head pitch (μm)	-0.96±0.87	2.07±0.86	0.19±0.45	-0.87±0.79	-0.94±0.52	-0.57±0.55
Degree of sperm-nucleus damage (%)	10.19±2.83	4.15±2.50	2.30±2.95	1.03±2.58	2.72±2.94	0.42±2.94

Data are presented as mean±SEM.
* $p<0.05$ vs control group.

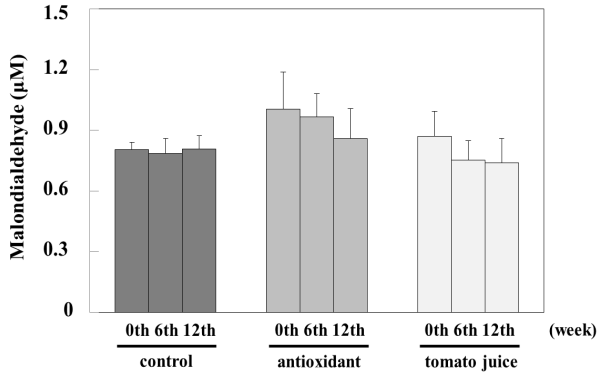

Figure 2. Semen plasma malondialdehyde levels in the three groups. Data are presented as mean±SEM.

group: 6) because of a freezer problem. Decreasing trend with time was observed in the antioxidant and tomato juice groups, but was not statistically significant.

Changes in semen parameters of each experimental group are shown in Table 2. There was no statistically significant difference in the parameters for antioxidant or tomato juice groups compared to the control group.

Changes in sperm parameters from the beginning of the experimental period are summarized in Table 3. In the tomato juice group, the amount of decrease of seminal plasma WBCs was statistically significant, compared to the control group at the 12th week (p=0.039); an increase in sperm motility was also statistically significant, compared to the control group at the 6th week (p=0.019). In the tomato juice and antioxidant groups, the amount of decrease of semen volume was statistically very significant, compared to the control group at the 12th week (tomato juice group vs control group; p=0.037, antioxidant group vs control group; p=0.035).

DISCUSSION

Oxidative stress with excessive generation of reactive oxygen species (ROS) may play an important role in male infertility.[5] A prospective study demonstrated that men with higher ROS generation had only about a 15% chance of pregnancy, compared to men with low ROS.[16] Although which reactive oxygen plays a critical role in oxidative damage in the sperm is unknown, Griveau et al proposed in an in vitro study that singlet oxygen intervened in the lipoperoxidation process in human spermatozoa.[19] Lycopene is a major carotenoid in tomatoes and is one of the most efficient singlet oxygen quenchers.[11] We therefore hypothesized that regular consumption of tomato juice rich in lycopene would potentially result in benefits to male fertility by enhancing antioxidant capacity in sperm. To investigate this hypothesis, we set seminal plasma lycopene levels and sperm parameters as the main endpoints of this study in infertile men. We observed a significant increase in seminal plasma lycopene levels after consumption of tomato juice for 12 weeks. Many reports have shown that consumption of tomatoes and tomato products increases lycopene levels in various human biological fluids, such as blood[20] and breast milk,[21] but there are few reports on an increase in seminal plasma.[22] In our study, seminal plasma lycopene level at the 12th week was higher than that at the 6th week; the rate of lycopene accumulation in seminal plasma is lower than in blood.[23]

ROS are hypothesized to affect sperm function through peroxidation of polyunsaturated fatty acids in the sperm plasma membrane.[5,6] MDA is an important marker of seminal plasma peroxidation. Seminal plasma MDA levels were significantly higher in infertile compared to fertile men.[24,25] Moreover, seminal plasma MDA level was negatively correlated with sperm motility and counts in fertile and infertile men. In our study, we could not determine the effects of antioxidants or tomato juice on seminal plasma MDA levels because of inadequate sample size. Further investigation is needed to elucidate the effects.

Consumption of tomato juice decreased semen WBCs in the present study. The WHO defines leukocytospermia as semen WBCs >10^6/mL. Although the association between semen WBCs and quality is still a matter of debate in the literature,[26] many studies reported that semen WBCs negatively affect semen quality as a result of ROS produced by WBCs.[27-29] In this study, the effects of a decrease of semen WBCs on semen quality in the tomato juice group were uncertain, because the baseline value was in the normal range (0.63±0.08×10^6/mL).

We recruited subjects with low sperm concentration (<20×10^6/mL) or low sperm motility (<50%). After regular consumption of tomato juice, there was no statistical change in sperm concentration, but there was significant improvement in sperm motility at the 6th week. Various authors recognize semen parameters such as sperm motility, concentration, and morphology as vital for assessment of fertility. Semen parameters were also correlated with in vitro oocyte fertilization rates.[30-32] Our results indicated that tomato juice consumption for 6 weeks may have a positive effect on asthenozoospermia by improving sperm motility. However, the improvement was not observed at the 12th week in the same group. This might be dependent upon the large variation in sperm motility within an individual, which is influenced by many factors, such as a period of sexual abstinence. The usefulness of tomato juice consumption for sperm motility should be evaluated in further prospective studies.

Gupta et al[15] reported that consumption of 2 mg of lycopene twice a day improved sperm motility in men with idiopathic nonobstructive oligo/astheno/teratozoospermia. The amounts of vitamin C (38 mg) and vitamin E (3 mg) in the can of tomato juice that we used were much smaller than those in the antioxidant pills (vitamin E 200 mg/day, vitamin C 200 mg/day, glutathione 400 mg/day) in this study. This suggests that lycopene is the active ingredient in tomato juice that improves sperm motility.

In our study, the semen volume was decreased at the 12th week in both the antioxidant and tomato juice groups. There are many reports that antioxidant intake either improves or has no effect on the semen volume, but no reports show decreases in these parameters. Semen volume is affected by daily habits, such as smoking[33] and cholesterol intake.[34] In this study, the subjects were not proscribed any daily activities, except for eating lycopene-rich foods, including tomatoes, in the feeding period. Therefore, we could not determine the effects of lifestyle, but these may be a factor, rather than consumption of

tomato juice and intake of antioxidant pills.

Antioxidant compounds (vitamins, glutathione, ubiquinol, and carnitine alone) were administered to male infertile patients, because of positive effects on semen parameters.[35,36] In a small trial in 14 infertile men who received the same three antioxidants as in this study (vitamin E 200 mg/day, vitamin C 200 mg/day, glutathione 400 mg/day), there were significant positive effects on sperm concentration.[37] Meanwhile, there are many reports that consumption of antioxidants has no effect on semen quality, so the effect of this antioxidant treatment on sperm quality is still an open question.[38,39] Moreover, the mechanisms by which antioxidant treatment affect sperm quality are not yet known. It was reported that many antioxidants, like vitamin E, vitamin C, and glutathione, were present in seminal plasma.[40-42] However, whether these antioxidants accumulate in the sperm after long-term oral intake is still unclear.

In the present study, intake of antioxidants (vitamin E, vitamin C, glutathione) yielded no statistical change in semen parameters, except for sperm volume. To confirm the effects of antioxidant pills on male infertility, more research is needed.

Conclusion

In conclusion, regular consumption of tomato juice seems to improve sperm motility in infertile patients. This is the first report to show that commercially available food, such as tomato juice, might benefit male infertility. The active ingredient in tomato juice that improves sperm motility may be lycopene, but the mechanism is still unknown. To confirm the effect of tomato juice in detail, we are now planning a large-scale interventional study.

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AUTHOR DISCLOSURES

The authors declare that they have no competing interests.

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