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Alpha- and gamma-tocopherol levels in human semen and their potential functional implications

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Abstract

Vitamin E is a potent antioxidant that has been considered involved in fertility, but studies have mostly focused on α-tocopherol. Our study aimed at measuring, by an isotope dilution gas chromatography–mass spectrometry method, α- and γ-tocopherol concentration in human semen in a large and well-characterised population (134 men with different semen parameters and in varicocele patients), as well as their potential role in male fertility. We carried out freeze/thaw experiments in 15 samples with the two isomers in the cryoprotective medium. Moreover, our study included 10 subjects supplemented in vivo with α-tocopherol for 90 days. In seminal plasma, γ-tocopherol concentration was significantly lower in the varicocele group than in the normozoospermic group. We observed that γ-tocopherol, supplemented to cryopreservation medium, induced a higher post-thaw human sperm viability and motility than α-tocopherol. The results of in vivo α-tocopherol supplementation showed a decrease in γ-tocopherol concentration with increasing α-tocopherol level in blood. This is the first report related to γ-tocopherol distribution in human semen analysed by gas chromatography–mass spectrometry. γ-tocopherol would not seem to be related to semen parameters but to cellular oxidative condition. This tocopherol may contribute to human health in a yet unexplored way.

KEYWORDS

GC-MS analysis, male fertility, oxidative stress, sperm cryopreservation, vitamin E

1 | INTRODUCTION

Oxidative stress, resulting from the imbalance production of free radicals and their removal by antioxidants, is considered a prominent cause of male infertility by affecting the quality and function of spermatozoa. It is estimated between 30% and 80% of men with infertility have high levels of reactive oxygen species (ROS) in semen (Ko, Sabanegh, & Agarwal, 2014; Makker, Agarwal, & Sharma, 2009; Tremellen, 2008; Zini, San Gabriel, & Baazeem, 2009). However, a controlled production of ROS is required for the maturation, capacitation and acrosome reaction of the spermatozoa and, therefore, for natural fertilisation (Aitken, Gibb, Baker, Drevet, & Gharagozloo, 2016; Griveau & Le Lannou, 1997; de Lamirande & Lamothe, 2009).

Spermatozoa are particularly susceptible to oxidative damage, given the massive presence of polyunsaturated fatty acids in their plasma membranes (Zerbinati et al., 2016).

Lipid peroxidation can alter membrane fluidity and integrity, provoking increased permeability and impaired motility of spermatozoa, and inhibit membrane fusion events (acrosome reaction and sperm–oocyte interaction) and viability (Potts, Notarianni, & Jefferies, 2000; Taylor, Roberts, Sanders, & Burton, 2009).

Chiara Zerbinati and Luisa Caponecchia are contributed equally to this study.

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Under physiological condition, the human semen contains diverse enzymatic antioxidants—that is superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and low molecular weight antioxidants, that is. ascorbic acid and α-tocopherol (α-TOH), and chelators of transition metals, that is transferrin, lactoferrin and ceruloplasmin, which constitute the total load of the antioxidant capacity of spermatozoa (Kefer, Agarwal, & Sabanegh, 2009). Vitamin E is one of the most investigated and powerful antioxidant. The term 'vitamin E' is inclusive of four stereoisomers namely α-, β-, γ- and δ-tocopherol (Zerbinati, Galli, Regolanti, Poli, & Iuliano, 2015). These vitamers are characterised by different number and position of methyl groups in the chroman ring and found in biological fluids in different concentrations, of which the rank order is approximately $\alpha \gg \gamma \gg \beta \approx \delta$ (Podda, Weber, Traber, & Packer, 1996). Some beneficial effects of Vitamin E have been highlighted in many aspects of human health (Galli et al., 2012; Micheletta et al., 2004), including male fertility (Bisht, Faiq, Tolahunase, & Dada, 2017; Kessopoulou et al., 1995; Suleiman, Ali, Zaki, el-Malik, & Nasr, 1996). Generally, four approaches have been used to evaluate the effects of vitamin E on male infertility: measurement of tocopherols in semen; in vitro and in vivo effects of vitamin E supplementation; vitamin E as additive to cryopreservation media.

Many studies have mostly focused on α-tocopherol, as it is the most abundant form in blood and tissues and it can be easily measured by HPLC with UV detector (Greaves, Jolly, Woollard, & Hoad, 2010; Lazzarino et al., 2017; Moretti et al., 2011). In contrast, γ-tocopherol (γ-TOH) has been poorly investigated—due to analytical limitations, that is its concentration in blood and tissue is below the detection limit of routine HPLC, despite its potential bioactivity (Devaraj & Traber, 2003).

The present study aimed at measuring the isomers α- and γ-TOH by gas chromatography–mass spectrometry (GC-MS) in human semen from normozoospermic, oligoasthenotheratozoospermic, asthenozoospermic and varicocele patients. In addition, we investigated the potential effects of these two vitamers on frozen/thawed semen fluids, based on the knowledge that oxidative stress induced by cryopreservation affects sperm quality. We also quantified tocopherols concentrations on blood plasma and semen from patients undergoing oral therapy with α-TOH to study variations in analyte concentrations in the peripheral circulatory system and in the testicular compartment.

2 | MATERIAL AND METHODS

2.1 | Study population and design

To investigate the profile of human sperm vitamin E isomers in subjects with normal and altered sperm characteristics, we recruited 168 subjects who undergone basic sperm analysis between January 2012 and June 2014 at the Centre of Andrology of S.M. Goretti Hospital (Latina). In order to investigate the possible correlation to oxidative stress and semen vitamin E, we included a group of patients with varicocele, given the reportedly relationship between varicocele and oxidative stress (Agarwal, Hamada, & Esteves, 2012; Ferramosca, Albani, Coppola, & Zara, 2015). Varicocele was diagnosed by scrotal colour-Doppler ultrasound during rest and Valsalva manoeuvre. According to physical examination, the varicocele was graded as grade ≥ II.

Semen analysis was performed in all patients. All participants gave their signed informed consent for authorising that part of their semen could be used for the study.

The study procedure was run in accord with the guidelines of the referring Ethical Committee, which approved the protocol, and the Helsinki Declaration of 1975. The study was registered at clinicaltrials.gov with identifier # NCT02062229.

2.2 | Semen analysis

Semen samples were obtained by masturbation into a sterile container after 3–5 days of sexual abstinence. After 60 min of liquefaction at 37°C, all samples were analysed by the same biologist (LC). Semen analysis was performed by light microscopy according to the World Health Organization guidelines (WHO, 2010), taking into account the following variables: ejaculate volume (ml), total sperm number (x 10^6/ejaculate), progressive motility (%) and morphology (% abnormal forms). Sperm concentration was examined in a Mackler chamber (Sefi Laboratories). Nine semen samples containing >1 × 10^6 leucocytes/ml were excluded from the study, because leucocytes are recognised as a major source of ROS in semen (Ochsendorf, 1999). To provide evidence for compliance with WHO recommendations, our andrology laboratory participates in an accreditation programme of the Italian Society of Andrology and Sexual Medicine (SIAMS).

2.3 | α-TOH and γ-TOH GC-MS analysis

We investigated semen samples from 134 participants, aged 34.5 ± 7.5. Subjects were allocated into four groups according to sperm parameters: Group 1, ≥50th percentile (33 normozoospermic men); Group 2, ≤5th percentile (32 oligoasthenoteratozoospermic men); Group 3 only progressive motility ≤ 5th percentile (25 asthenozoospermic men); and Group 4, 44 patients with varicocele. An aliquot of semen was stored at −80°C until GC/MS analysis. α- and γ- TOH were measured by GC-MS using deuterium-labelled internal standards as previously described (Zerbinati et al., 2015). In brief, 200 μl of semen was extracted with 1 ml of hexane, the solvent was evaporated under a stream of nitrogen and incubated for derivatisation with hexamethyldisilazane, trimethyldichlorosilane and pyridine (3:1:9) (Supelco) at 60°C for 30 min. After incubation, the mixture was evaporated under a flux of nitrogen, and the residue added with n-hexane into an autosampler vial. GC-MS analysis was performed with an Agilent 5973N system (Agilent). Samples were automatically injected

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into an HP-5MS capillary column (30 m, 0.25 mm ID, 0.5 mm film thickness). The following molecular ions were monitored with the mass spectrometer operating in selected ion mode: [$^2H_3$] α-TOH 505.5 m/z, α-TOH 502.5 m/z, [$^2H_2$] γ-TOH 490.5 m/z and γ-TOH 488.5 m/z.

2.4 | Sperm cryopreservation

We recruited 15 patients to collect sperm samples and test the potential protective effects of the two vitamin E isomers in spermatozoa exposed to freeze–thawing cycles mimicking the processing of spermatozoa in fertility clinics. Samples from normozoospermic men (age 31.7 ± 7.1) were cryopreserved and used for freeze–thaw experiments.

After sperm liquefaction, which was performed by incubation at 37°C for 60 min, the ejaculate was divided into three fractions and each fraction was drop wise added, with gentle stirring, an equal volume of sperm freezing medium (Quinn's advantage sperm freezing medium, Sage) supplemented with Ethanol (control samples) or α-TOH 120 μM (f.c.) or γ-TOH 120 μM (Sigma-Aldrich). Samples were equilibrated for 10 min at 37°C, after then transferred to straws (CBS High Security Sperm Straw, Cryo Biosystem) and subjected to nitrogen vapours for 8 min and finally submerged in liquid nitrogen.

To verify whether spermatozoa tolerated tocopherols exposure, sperm viability test was carried out before the cryopreservation experiments. Cell death was evaluated by eosin Y (0.5%) staining, counting 200 spermatozoa per sample at 400x magnification. The amount of living spermatozoa was expressed as a percentage of total counted cells (WHO, 2010). Concentrations up to 120 μM α-TOH and γ-TOH did not affect spermatozoa viability (data not shown).

Thawing procedure. The cryopreserved aliquots of the three fractions were thawed after a week. The straw was kept at room temperature and after 15 min samples added with sperm washing medium (Quinn's advantage medium with HEPES, Sage), and the spermatozoa pelleted at 300 g for 10 min. The sperm pellet was resuspended with 100 μl of sperm washing medium and was immediately processed for viability and motility.

Sperm motility was assessed by automated computer-assisted analysis (Sperm Class Analyzer Software, SCA, Microptic Co.) on each aliquot after thawing. At least 10 visual fields were captured, and at least 400 spermatozoa were counted in each determination. The variables taken into consideration were total motility (%), progressive motility (%), curvilinear velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), linearity (LIN, %), amplitude of lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz).

2.5 | In vivo α-TOH administration

We studied the effect of in vivo α-TOH administration to evaluate vitamin E isomers concentration on semen and blood plasma. Ten infertile patients, ageing 34.9 ± 8.6 (mean ± *SD*), were selected for the study because they met the following inclusion criteria: no medical treatment in the previous three months, no smoking habit, absence of systemic or andrological diseases. Patients were supplemented with 800 IU α-tocopherol (Rigentex, Bracco Spa) for three months. Blood and sperm samples, for tocopherol analysis, were obtained at baseline (before starting α-tocopherol supplementation, T0), after 45 (T1) and 90 (T2) days of treatment. Patient adherence to supplementation and any potential side effects were also recorded.

2.6 | Statistical analysis

A descriptive analysis was conducted to show the characteristics of the 134 subjects. Data were summarised as mean, standard deviation (*SD*) or standard error of mean (*SEM*), median and interquartile range, or frequencies and percentages as appropriate. Comparisons among groups were made using one-way ANOVA. Tukey's multiple comparison post hoc test was then used to determine significant differences between the groups. All statistical analyses were conducted using SPSS version 21 software (IBM Corporation).

3 | RESULTS

Data on demographic and semen analysis of 134 subjects enrolled in the study are those previously reported in Zerbinati et al. (2016). The four groups did not differ in terms of ejaculate volume. As expected from the enrolling procedure, semen parameters were significantly different between the four groups. Patients in varicocele group were younger than those in the other groups. Smoking habits were not significant among the analysed groups.

We first measured α-TOH and γ-TOH concentration in the whole population of patient to establish values of the two vitamers

TABLE 1 Comparison between α-TOH and γ-TOH concentrations in patients

		Group 1	Group 2	Group 3	Group 4	P value
α-TOH	μg%	19.51 ± 11.84	13.81 ± 10.33	16.39 ± 11.73	14.56 ± 11.25	n.s.
γ-TOH	μg%	0.27 ± 0.26	0.21 ± 0.41	0.28 ± 0.43	0.14 ± 0.10	<.0001*

Note: Group 1, normozoospermic; group 2, oligoasthenoteratozoospermic; group 3, asthenozoospermic; group 4, varicocele.
Values are expressed as mean (±*SD*). Comparision among groups by one-way ANOVA
*1 versus 4, $p < .0001$

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according to the WHO classification of patients. The comparison between α-TOH and γ-TOH concentrations in all groups is shown in Table 1. α-TOH concentration in semen was almost two orders of magnitude higher than γ-TOH.

We did not find any statistical difference in α-TOH concentration between the groups. In contrast, γ-TOH concentration was significantly lower in varicocele group than in the normozoospermic group ($p < .0001$).

Semen parameters of the 15 patients enrolled for freeze–thaw experiments with α-TOH and γ-TOH are summarised in Table 2. Post-thawing sperm viability and motility were assessed by eosin test and computer-assisted analysis.

γ-TOH treated samples were associated with 27% higher viable spermatozoa compared to control samples (Figure 1).

No kinetic parameters of spermatozoa were affected by treatment with α-TOH compared to control samples. In contrast, samples treated with γ-TOH resulted in significant better performance in total and progressive motility, VCL and VSL (Figure 2).

In the in vivo study, we analysed α- and γ-TOH concentration in blood and semen before and after 45 and 90 days of α-TOH supplementation (Figure 3).

A significant increase in blood α-TOH was observed after 45 days (88%) and 90 days (37%). In contrast, γ-TOH in blood fell by 33% after 45 days of α-TOH supplementation. In response to supplementation, semen α-TOH increased by 38% after 45 days, while no changes were found in γ-TOH.

We did not observe significant differences in any semen parameter after supplementation (data not shown).

No side effects were reported by patients taking the supplement.

4 | DISCUSSION

Human semen contains several antioxidants and vitamin E is one of the most represented. While a complete profile of tocopherol isoforms has been reported in animal studies (Gliozzi et al., 2009; Mourvaki, Collodel, Moretti, Cosci, & Castellini, 2008), only few studies concerning human semen, based on HPLC methods, measured TOH concentration, focusing mainly on α-TOH (Benedetti et al., 2012; Lazzarino et al., 2017; Moretti et al., 2011).

We investigated the distribution of different tocopherol isomers in semen of a large population with different semen parameters and report the first study α-TOH and γ-TOH in human semen by a specific and sensitive isotope dilution method.

There were not significant differences in α-TOH concentrations among the patients of our population, in accord with some previously published studies (Lewis, Sterling, Young, & Thompson, 1997; Moretti et al., 2011). However, Omu, Fatinikun, Mannazhath, and Abraham (1999) reported that α-TOH concentration is higher in blood than in semen and that concentration was higher in samples of men with normal semen parameters compared samples with abnormal parameters. This discrepancy could be due to the low number of analysed patients, to the selection criteria, to the method of analyses used and to the assay performed only on seminal plasma. Lewis et al. (1997) analysed α-TOH concentration in seminal plasma of 18 normozoospermic fertile men, 20 normozoospermic infertile men and 21 asthenozoospermic infertile men and did not find significant differences among these groups. Moretti et al. (2011) selected 27 patients with inclusion criteria similar to those used here; 12 patients had one or more altered semen parameter and 15 were normozoospermic patients. These two groups presented comparable α-TOH levels. Al-Azemi, Omu, Fatinikun, Mannazhath, and Abraham (2009) showed an association between low semen α-TOH concentration and altered sperm function in 40 patients with different seminal parameters. However, the authors considered only obese men with an average BMI of about 35, which is known to affect the antioxidant status (Kilic et al., 2016; Ozata et al., 2002). Our patients had a BMI < 30 kg/m^2 and a comparable smoking status.

In our study, γ-TOH would not seem to be related to semen parameters but to cellular oxidative condition, in fact we found a significantly lower concentration of this tocopherol isomer in the varicocele group compared to normozoospermics. Our data are in agreement with those reported by Gvozdjakova et al. (2013) who found no statistically

TABLE 2 Semen characteristics of 15 patients before freeze–thaw experiments

	n = 15
Age	31.7 (±7.1)
Ejaculate volume (ml)	3.9 (±1.4)
Spermatozoa/ml (×10^6)	80.0 (60.0/130.0)
Spermatozoa/ejaculate (×10^6)	325.5 (156.0/650.0)
Progressive motility (%)	50.0 (40.0/60.0)
Atypical forms (%)	74.0 (72.0/77.0)
Leucocyte (×10^6)	0.6 (±0.35)
Vitality test (%)	66.9 (±8.6)

Note: Values are expressed as mean (±*SD*), median (min/max) or frequencies (percentage).

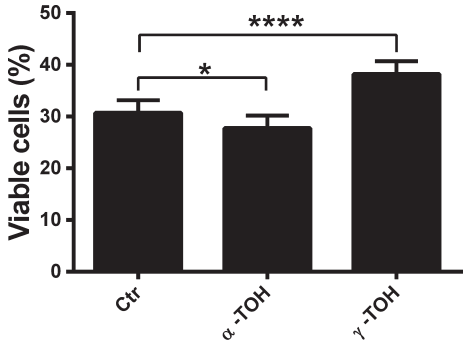

FIGURE 1 Effect of α-tocopherol and γ-tocopherol on viability of spermatozoa subjected to cryopreservation. Viability was assessed after thawing samples supplemented with ethanol (Ctr, control group), α-tocopherol (α-TOH, 120 μM) or γ-tocopherol (γ-TOH, 120 μM). Data represent mean ± *SEM* of three separate experiments; *$p < .05$ versus control group; ****$p < .0001$ versus control group

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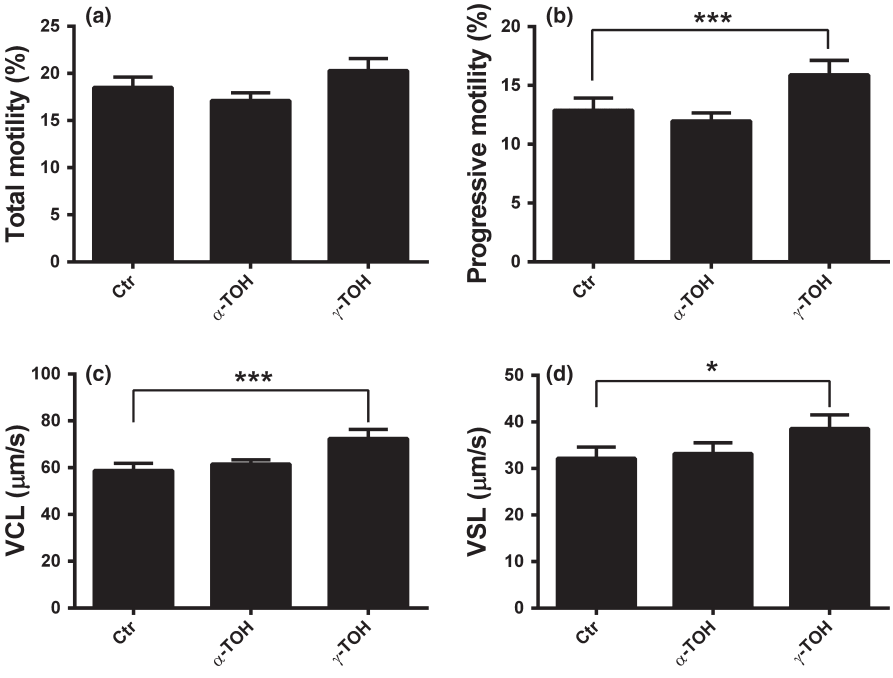

FIGURE 2 Effect of α-tocopherol and γ-tocopherol on kinetic parameters of spermatozoa subjected to cryopreservation. Automatic computer-assisted analysis of total motility (a), progressive motility (b), curvilinear velocity (VCL) (c) and straight-line velocity (VSL) (d) in samples supplemented with ethanol (Ctr, control group), α-tocopherol (α-TOH, 120 μM) or γ-tocopherol (γ-TOH, 120 μM). Data represent mean ± *SEM* of three separate experiments; ***$p < .001$ versus control group; *$p < .05$ versus control group

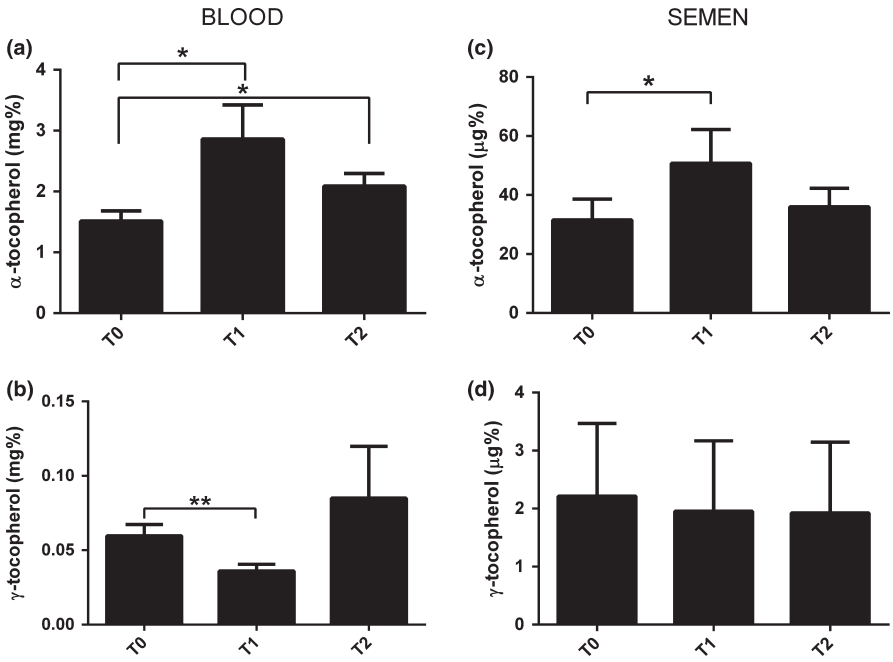

FIGURE 3 α-tocopherol and γ-tocopherol concentrations in blood and semen after in vivo α-tocopherol administration on 10 patients. α-tocopherol (α-TOH) or γ-tocopherol (γ-TOH) concentrations in blood (a, b) and in semen (c, d) at basal time (T0), 45 days (T1) and 90 days (T2) of therapy; *$p < .05$; **$p = .001$

significant difference in the levels of γ-TOH concentration in 37 patients grouped into two categories of motility. Benedetti et al. (2012) measured γ-TOH concentration in blood and semen plasma samples of 31 infertile and 12 fertile males and showed that seminal plasma γ-TOH concentration was significantly lower in infertile men than in fertile subjects. This work cannot be compared to the present one because the patients were not grouped according to semen parameters, and the evaluation of γ-TOH concentration was limited to seminal plasma.

γ-TOH has long been considered of minor importance compared to α-TOH due to its lower plasma concentration. Some evidence suggested many biological roles for γ-TOH and the control of the nitric oxide (NO)-related toxicity is included among these (Jiang, Christen, Shigenaga, & Ames, 2001). All vitamin E forms are powerful antioxidants in relation to lipid radicals. γ-TOH is very efficient in scavenging nitrogen dioxide resulting in a stable nitro adduct, 5-nitro- γ-tocopherol. α-TOH does not have this capacity because of C5 is functionally closed by a methyl group (Jiang et al., 2001).

Moreover, γ-TOH could have a similar efficiency of, or even more potent than α-TOH in inhibiting lipid peroxidation in vitro at temperature higher than 35°C (Hove & Hove, 1944). The mechanism of action of ROS involves lipid peroxidation of the sperm plasma membrane, which is highly susceptible to oxidative damage due to large

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amounts of polyunsaturated fatty acids, by impairing membrane fluidity and mobility (Aitken et al., 2016).

Compared with α-TOH, γ-TOH exhibits slightly stronger radical scavenging activity. Spermatozoa spontaneously produce a variety of ROS, including NO, and in varicocele patients an increased generation of NO from endothelial cells can further deteriorate the balance towards oxidative stress (Agarwal et al., 2012; Buzadzic et al., 2015). We showed that γ-TOH concentration is lower in patients with varicocele than those in the other groups. Probably, this depends upon the consumption after trapping NO to form 5-Nγ-T.

Increased ROS production could occur in varicocele by infection/inflammation (Henkel, 2011), ageing (Sastre, Pallardó, García de la Asunción, & Viña, 2000), and accelerated by freeze-thawing (Chatterjee & Gagnon, 2001).

Notwithstanding several measures to improve sperm cryo-survival, a reduction in motility of 25%-75% is commonly observed after thawing (Kalthur, Adiga, Upadhya, Rao, & Kumar, 2008; O'Connell, McClure, & Lewis, 2002). Some studies reported on the effect of supplementation of α-TOH in the freezing medium, but none used γ-TOH. We report that γ-TOH is able to maintain a higher post-thaw human sperm viability and progressive motility than α-TOH. In studies on dogs and boars, α-TOH supplementation increased post-thawing forward sperm motility (Michael et al., 2007), the capacitating conditions (Satorre, Breininger, Beconi, & Beorlegui, 2009), and may protect sperm from oxidative stress by targeting the expression of apoptosis-related genes (Jeong et al., 2009). No difference was found in sperm viability between samples frozen in the presence or absence of α-TOH. In addition, in a human study no significant improvement was observed in post-thaw viability by adding α-TOH (Taylor et al., 2009).

Kalthur et al. (2011) reported that 5 mM α-TOH supplementation to cryopreservation medium increased the post-thawing sperm motility and protected DNA integrity in 38 normozoospermic and 59 asthenozoospermic patients, regardless of basal semen parameters. In contrast, by using α-TOH we did not observe significant improvement in post-thawing motility, but a cautionary note is needed before drawing conclusions because we use a lower concentration.

On the other hand, Askari, Check, Peymer, and Bollendorf (1994) showed that vitamin E scarcely improved the post-thaw motility by using 10 mM α-TOH. In another study, the authors evaluated the effect of α-TOH on post-thaw sperm motility by supplementing normal and abnormal samples. These authors showed a significantly improvement in post-thaw motility using 200 μM of α-TOH (Taylor et al., 2009). In our study, samples treated with γ-TOH resulted in better performance in post-thawing total and progressive motility than samples treated with α-TOH.

Many animal studies in preclinical models and few in vivo human studies have been reported to examine the potential benefits of γ-TOH to treat a variety of disease (Jiang et al., 2001). However, to our knowledge, no studies have been carried out in the male fertility setting using γ-TOH in vivo supplementation and, at present, there is no γ-TOH supplement available for human use. Most of the studies used the alpha isomer mixed with other antioxidants (Ahmadi, Bashiri, Ghadiri-Anari, & Nadjarzadeh, 2016). A few supplementation studies, using specific α-TOH preparations, were addressed to the evaluation of semen parameters. Kessopoulou et al. (1995) conducted a randomised, double blind trial on 15 treated patients and 15 placebo patients. The authors administered either 600 mg/day of vitamin E or identical placebo tablets for 3 months and found no significant improvement in any of the conventional semen parameters. In another randomised double bind trial on 52 patients treated with 400 mg/day of vitamin E and 35 with placebo for 6 months, the authors showed a significant increase in motility and in pregnancy rate in treated group, compared to the control group (Suleiman et al., 1996). A recent study examined the effectiveness of vitamin E alone on semen parameters of 30 patients with oligoasthenozoospermia. After 6 months treatment of 400 mg/day Vitamin E, the study revealed a significant improvement in sperm motility (ElSheikh et al., 2015).

Our results did not show significant difference in any of the semen parameters. In this context, apart from dosage the duration of treatment should be taken into account too.

While considering the limitation of translating the in vitro data to in vivo efficacy, we wanted to look at the two isomer concentrations (α-TOH and γ-TOH) in blood plasma and in semen after in vivo tocopherol administration. On the one hand, we could not test the effect of γ-TOH in vivo because it was not available as a supplement. On the other hand, it is known that α-TOH supplementation can lead to a reduction of γ-TOH in blood (Handelman, Machlin, Fitch, Weiter, & Dratz, 1985).

In accord, we observed that supplementation of α-TOH depletes blood of γ-TOH. Therefore, we infer that the decrease in γ-TOH levels after α-TOH supplementation is potentially deleterious and that oral supplements of vitamin E should contain combination of α-TOH and γ-TOH, to avoid the replacement of γ-TOH by α-TOH in lipid membranes, resulting in decreased blood plasma γ-TOH levels. This effect is probably due to the hepatic α-TOH transfer protein, which preferentially incorporates α-TOH into the plasma, as well as increasing hepatic catabolism of γ-TOH to γ-carboxyethyl-hydroxychroman (γ-CEHC) (γ-TOH water-soluble metabolite) (Devaraj, Leonard, Traber, & Jialal, 2008). Finally, it must be considered that α-TOH, as synthetic isomer of vitamin E, has been reported to act as a co-carcinogen in in vitro model systems (Vivarelli et al., 2019) in support of the hypothesis that α-TOH might sustain prostate tumour growth (Klein et al., 2011).

5 | CONCLUSION

This is the first report related to γ-TOH distribution in human semen analysed by GC-MS.

γ-TOH would not seem to be related to semen parameters but to cellular oxidative condition. Although α-TOH is considered the most important component of vitamin E, γ-TOH may significantly contribute to human health by specific antioxidant actions. The use of large

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doses of α-TOH leads to undesired depletion of γ-TOH in blood and accumulating evidence suggests that γ-TOH has unique properties and that is relevant to prevention and therapy against chronic disease (Jiang, 2014).

Controlled studies in human fertility are required to establish the potential benefits of γ-TOH and our findings open the investigation about γ-TOH in the setting of cryoprotective media.

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