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ORIGINAL ARTICLE

Altered Semen Quality is Associated with Decreased Semen Docosahexaenoic Acid and Increased Oleic Acid Levels

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SUMMARY

Background: **Fatty acids composition of the spermatozoa may be an important determinant of sperm quality and fertility. This study aimed to evaluate the fatty acids profile of seminal plasma and membrane spermatozoa and to study the association between fatty acids and sperm properties.**
Methods: **Semen samples were collected by masturbation from 45 middle-aged men consulting for infertile couples. Semen cytomorphological analysis was performed after liquefaction. Semen was classified as normal or abnormal according to World Health Organization criteria 2010. Plasma seminal and spermatozoa membrane fatty acids composition were analyzed by capillary gas chromatography.**
Results: **Docosahexaenoic acid level was decreased while oleic acid level and n-6:n-3 ratio were increased in spermatozoa membrane in men with abnormal sperm. However, no variation in seminal plasma fatty acid composition was found between men with normal and abnormal sperms. Spermatozoa docosahexaenoic acid was positively correlated with sperm concentration and progressive motility and inversely related to atypical spermatozoa number, while oleic acid showed the inverse correlations.**
Conclusions: **Altered fatty acids composition in the spermatozoa membrane, especially a decreased docosahexaenoic acid content, is associated with poor sperm quality. Although a causal association could not be established, intervention that recovers normal spermatozoa fatty acid composition could contribute to improved sperm quality.**
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KEY WORDS

fatty acids, gas chromatography, spermatozoa, semen

LIST OFABBREVIATIONS

ALA - alpha linolenic acid
ARA - arachidonic acid
DHA - docosahexaenoic acid
ASP - asthenozoospermia
ATSP - asthenoteratozoospermia
LA - linoleic acid
MUFA - monounsaturated fatty acids
NS - normal sperm
NSP - necrozoospermia
PA - palmitic acid

PUFA - polyunsaturated fatty acids
OA - oleic acid
OASP - oligoasthenozoospermia
OSP - oligozoospermia
SAFA - saturated fatty acids
TSP - teratozoospermia

INTRODUCTION

Polyunsaturated fatty acids (PUFA) are important constituents of cell membranes. They modulate membrane fluidity, permeability, and fusion, influencing transmembrane exchange, cellular communication, receptor recognition, and enzyme activity. Semen is one of the richest tissues in docosahexaenoic acid (DHA, C22:6n-3) [1-3] and semen fatty acid composition was shown to affect spermatozoa maturation, motility, and viability as well as fusion between the spermatozoa and the oocyte [4,5]. Alteration of fatty acid composition of SPZ membranes could perturb energy metabolism and membrane properties, which may affect sperm quality and function [6,7]. DHA was positively linked with sperm function. However, monounsaturated fatty acids (MUFA) were negatively linked to sperm concentration and motility and the relationship of saturated fatty acids (SAFA) with sperm characteristics was inconsistent [1-3,6,7]. Oxidative stress is considered a condition that alters spermatozoa functions and exerts negative impact on fertility in men [8]. Excessive susceptibility of spermatozoa to oxidative damage is probably related, at least in part, to their high PUFA content. This study aimed to explore fatty acid composition in seminal plasma and spermatozoa membranes in normal and abnormal sperm and look for the relationship between main fatty acids and selected sperm characteristics (i.e., concentration, mobility, vitality and morphology).

MATERIALS AND METHODS

Study population

Semen samples were obtained by masturbation on site from 45 men consulting for infertile couples. Participants with acute or chronic uncontrolled/severe illnesses (e.g., neoplasm, renal, liver, and heart failure), those with own or partners' condition that could account for infertility, and those with sperm ejaculates of low volume (< 1.5 mL), high content in leucocytes ($\geq 10^6$ cell/mL) or with positive bacteria result were not included. Infertility was defined as impossibility of conceiving after 12 months or more of regular unprotected sexual intercourse with the same partner [9]. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The protocol was approved by the Ethic Committee of Rabta Hospital and informed consent was obtained from all participants.

Sperm collection and preparation

Participants were encouraged to abstain from sexual relation and ejaculating for 4 days prior to sperm collection and to report the length of abstinence time. Samples were incubated for 30 minutes at 37°C for liquefaction and then divided into two aliquots for cytomorphological and biochemical analyses. Seminal plasma was obtained by centrifugation at 700 x g for 20 minutes at 4°C.

Cytomorphological analysis

Semen pH and volume were measured. Spermatozoa mobility was determined under a light microscope. Spermatozoa viability was evaluated under light microscopy after eosin-nigrosin staining. Spermatozoa were counted on a hemocytometer after immobilization using a formalized Ringer's solution (1/10 dilution) [10]. Semen abnormalities were defined according to World Health Organization criteria (WHO, 2010) as follows; oligozoospermia (OSP), number of spermatozoa < 15 million/mL; asthenozoospermia (ASP), progressive motility < 32%; necrozoospermia (NSP), living spermatozoa < 60%, and teratozoospermia (TSP), normal spermatozoa < 15%. Sperm with one or more abnormalities were classified as "abnormal sperm" and those with none of these abnormalities were classified as "normal sperm (NS)".

Biochemical analysis

Liquefied sperm were centrifuged at 700 x g for 20 minutes at 4°C. Seminal plasma was recovered and the spermatozoa pellet was washed twice with 9% sodium chloride, and 20 µL of butyl hydroxytoluene was added to the two specimens as an antioxidant and stored at -80°C until analysis. The FA profiles in seminal plasma and spermatozoa pellet were determined according to the method of Moser and Moser and modified by Nasrallah et al. [11]. Analysis was done on a HP6890 series II gas chromatograph (Agilent, Atlanta, GA, USA), equipped with a split/splitless capillary inlet system and a flame ionization detection (FID). Separation was performed on a polar capillary column (Innowax, 30 m x 0.25 mm x 0.25 µm) (Agilent) and nitrogen was used as the carrier gas at a flow of 1.2 mL/min and oven programmed temperature.

Statistical analysis

Statistical computations were performed using SPSS 18.0 for Windows software (SPSS Inc., Chicago, IL, USA). Continuous variables were tested for normal distribution using the Shapiro-Wilk test. Comparisons between groups were achieved using independent-samples *t*-test for continuous variables and chi-squared test for categorical variables. Relationship between continuous variables was tested using Pearson's correlation. A p-value < 0.05 based on two-sided calculation was considered significant.

Table 1. Demographic and semen characteristics and spermatozoa membrane fatty acid profile in study participants.

	Men with normal sperm (n = 16)	Men with abnormal sperm (n = 29)
Demographic and semen characteristics		
Age, years	33.5 (4.89)	37.2 (8.12)
Previous procreation, %	15.4	4.25
Tobacco smoking, %	46.2	55.6
Sexual abstinence, days	4.35 (1.35)	4.41 (1.91)
Ejaculate volume, mL	3.74 (1.12)	3.28 (1.61)
Semen pH	7.71 (0.27)	7.71 (0.32)
Low viscosity, %	0	4
Spermatozoa fatty acids (in percentages of weight)		
C16:0	41.8 (9.40)	40.4 (6.82)
C18:0	20.9 (10.3)	22.3 (10.1)
C18:1n-9	4.46 (2.43)	9.07 (5.44) **
C18:2n-6	4.79 (1.93)	5.22 (2.59)
C20:4n-6	2.47 (1.37)	2.26 (1.34)
C20:5n-3	0.43 (0.77)	0.45 (0.44)
C22:5n-3	0.76 (0.41)	0.68 (0.72)
C22:6n-3	17.1 (11.3)	11.0 (9.95) *
PUFA:MUFA ratio	5.20 (3.63)	2.77 (2.26) **
n-6:n-3 ratio	0.83 (0.55)	1.43 (0.97) *

Values are expressed as mean (standard deviation) or percentage, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids; * - p < 0.05; ** - p < 0.01.

Table 2. Variation of selected semen fatty acids in different spermatic pathologies reported in literature.

		ASP	OSP	ATSP
Nissen et al. [12], 1983	Increased Decreased			 DHA
Zalata et al. [6], 1998	Increased Decreased	SA, OA, LA DHA	SA, OA, LA DHA	
Conquer et al. [7], 1999	Increased Decreased	OA DHA		
Gulaya et al. [13], 2001	Increased Decreased	 DHA		
Aksoy et al. [1], 2006	Increased Decreased	SA, OA DHA	SA, OA DHA	
Tavilani et al. [14], 2007	Increased Decreased	SA, LA DHA		
Khosrowbeygi et al. [15], 2008	Increased Decreased	OA DHA		OA DHA
Present study	Increased Decreased	OA DHA	OA DHA	OA DHA

ARA - arachidonic acid, ASP - asthenozoospermia, ATSP - asthenoteratozoospermia, DHA - docosahexaenoic acid, LA - linoleic acid, OA - oleic acid, OSP - oligozoospermia, PA - palmitic acid, SA - stearic acid.

Table 3. Reported correlations between the main spermatozoa membrane fatty acids and selected semen characteristics.

	Semen concentration (10^6 cell/mL)		Semen motility (%)		Atypical spermatozoa (%)	
	Positive	Negative	Positive	Negative	Positive	Negative
Nissen et al., 1983 [12]	DHA		DHA			DHA
Zalata et al., 1998 [6]	DHA		DHA			DHA
Conquer et al., 1999 [7]	DHA		DHA			
Lenzi et al., 2000 [28]	DHA	LA, ALA	DHA			DHA
Gulaya et al., 2001 [13]	DHA		DHA	ALA		
Tavilani et al., 2006 [2]			LA, ARA, DHA			DHA
Aksoy et al., 2006 [1]		SAFA	LA, ARA	SAFA		DHA
Khosrowbeygi et al., 2008 [15]			ARA, DHA	PA	PA	ARA, DHA
Safarinejad et al., 2010 [29]	EPA, DHA					
Present study	ARA, DHA	PA, OA	DHA	PA, OA	PA, OA	DHA

ALA - alpha linolenic acid, ARA - arachidonic acid, DHA - docosahexaenoic acid, LA - linoleic acid, OA - oleic acid, PA - palmitic acid, SAFA - saturated fatty acids.

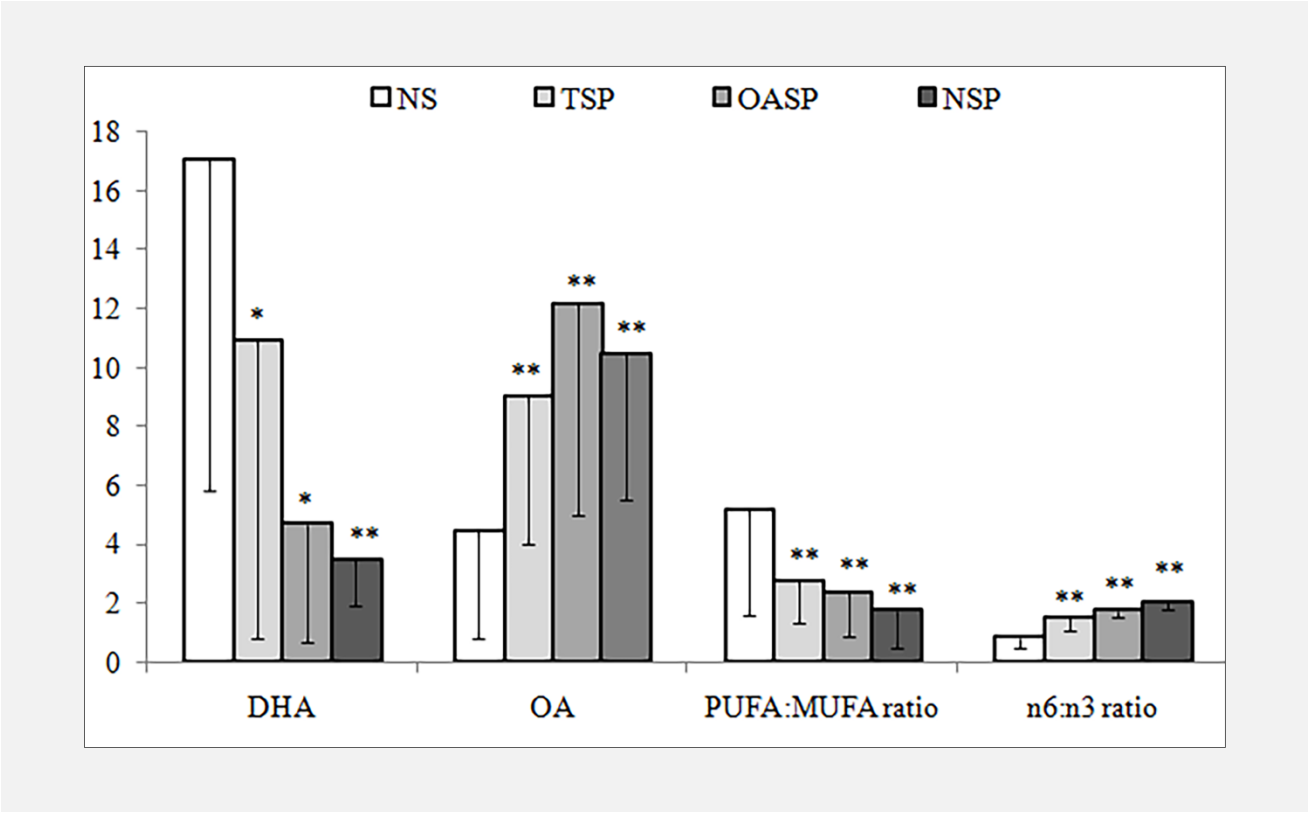

Figure 1. Spermatozoa membrane content in docosahexaenoic (DHA) and oleic (OA) acids (in percent weight) and fatty acids classes' ratios in diverse sperm abnormalities compared to normal sperm.

MUFA - monounsaturated fatty acids, NS - normal sperm, NSP - necrozoospermia, OASP - oligoasthenozoospermia, PUFA - polyunsaturated fatty acids, TSP - teratozoospermia, * - $p < 0.05$, ** - $p < 0.001$.

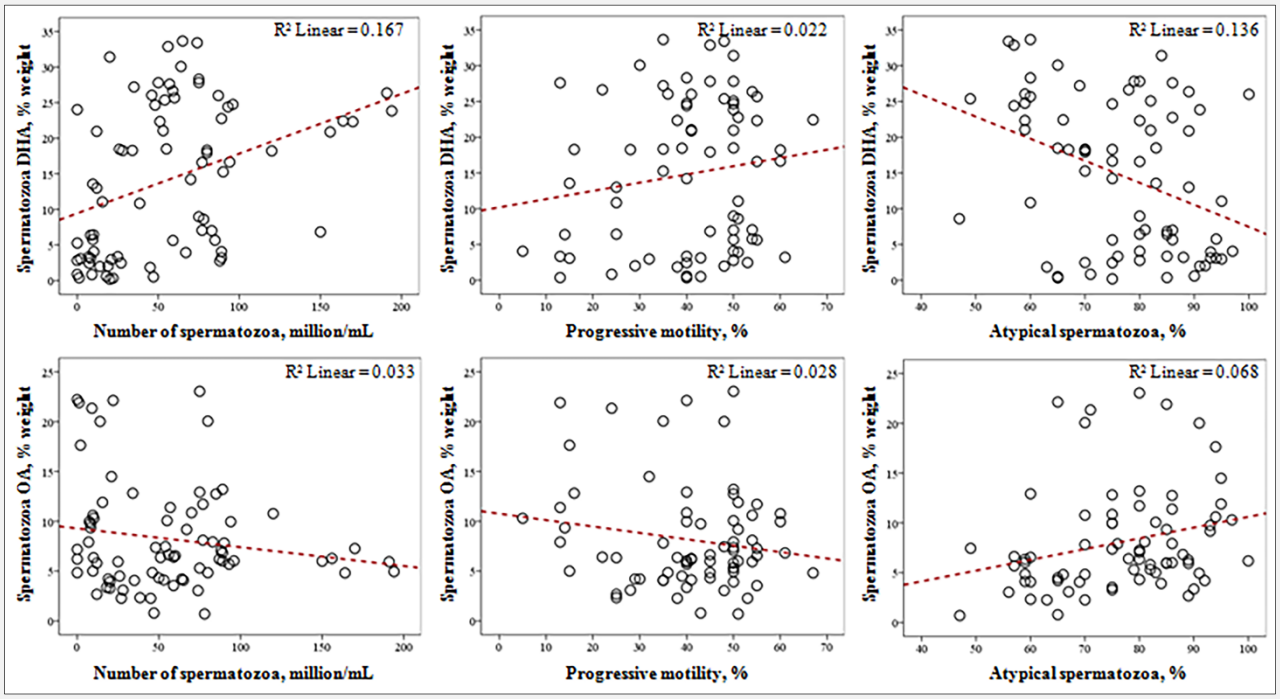

Figure 2. Correlations of spermatozoa membrane docosahexaenoic (DHA) and oleic (OA) acids with sperm concentration, motility, and atypical spermatozoa number.

RESULTS

Based on cytomorphological analysis, the sperm was considered normal in 16 participants and abnormal in 29 participants. More than half of participants (58%) showed TSP; around third of participants had OSP or ASP, and 13% of participants showed NSP. Demographic and semen characteristic in participants according to sperm quality are shown in Table 1. There was no significant difference in age, smoking, and previous procreation rate and sperm volume, pH and viscosity between the two groups.

No differences in seminal plasma fatty acid profiles were found between participants with normal or abnormal sperm. However, participants with abnormal sperm had significantly increased OA and decreased DHA contents in the spermatozoa membrane. The PUFA: MUFA ratio was decreased whereas the n-6:n-3 ratio was increased in those with abnormal sperm (Table 1). Similar findings (i.e., spermatozoa membrane decreased DHA and increased OA) are observed when considering individual or combined sperm abnormalities such as ASP, OSP, TSP, NSP or oligoasthenoteratospermia (Figure 1). In a combined group of normal and abnormal sperm, spermatozoa DHA was positively related to sperm concentration and progressive motility and inversely related to atypical spermatozoa number, while OA showed the inverse correlations (Figure 2).

DISCUSSION

This study showed altered fatty acid composition of spermatozoa membrane in sperm of poor quality with an increase in DHA and a decrease in OA contents. However, fatty acid composition in seminal plasma did not differ between men with normal or abnormal sperm. The findings are in accordance with previous studies that showed association between semen fatty acid profile and semen quality. Comparison between fertile and infertile men revealed a significant decrease in spermatozoa DHA content in participants with OSP, ASP or a combination of these anomalies [1,2,6,7,12-15]. A decrease of spermatozoa OA was reported in different spermatic pathologies [1,6,7,15] while no variation was found in semen OA of fertile and infertile men in other studies [2,12,13,16]. Sperm of poor quality was associated with increased semen PA and SA contents [1,6, 14], whereas other studies found no variation of these SAFA between normal and abnormal sperm [7,12,13, 15]. Semen LA level was found to be decreased [1,7], increased [13] or unchanged [1,7,12,13,15] in sperm of

poor quality (Table 2). The study showed that DHA correlates positively with semen concentration and motility and negatively with atypical spermatozoa number. However, PA and OA and n-6:n-3 ratio were inversely related to spermatozoa number and motility and positively related to atypical spermatozoa number. These findings are similar to literature data (Table 3).

Association between low DHA and high OA content of spermatozoa membrane and poor quality of sperm is understandable. It has been shown that OA and DHA apply contradictory effects on different properties of the spermatozoa. DHA in the membrane increases its fluidity [17], membrane flip-flop [18], the integration of proteins [19], and ability to merge with the oocyte during fertilization [20], whereas OA decreases these properties [21]. It has been shown that DHA intervenes in the trend of bending and flexing of flagellum of the spermatozoon and thus its motility [22]. On the other hand, PUFA are precursors of eicosanoids that can influence motility of the spermatozoon [4]. In contrast, OA has been shown to be toxic to the spermatozoa through a deterioration of seminal qualities such as decreased motility and vitality. Pellicer-Rubio and Combarnous [21] showed that OA is toxic to Capricorn spermatozoa decreasing their motility, quality of movement, and vitality. In addition, a high level of OA induces early acrosomal exocytosis and has a negative effect on motility regulation in Capricorn monkeys [23,24].

The association between sperm abnormalities and spermatozoa membrane fatty acid alteration raises the question of the causal link between the two conditions. Whether modified fatty acid composition is a cause, a consequence or epiphenomenon of poor sperm quality is unclear. Altered fatty acid composition in pathological sperm could be secondary to disturbed fatty acid dietary intake (i.e., high saturated and monounsaturated fat intake and unbalanced n-6 to n-3 PUFA intake) or altered fatty acid endogenous metabolism (i.e., reduced activity of enzymes elongase and desaturase). The change could be secondary to excessive degradation of long chain PUFA secondary to oxidative stress, which explains the decrease in DHA and the increase in OA into spermatozoa membrane. It was well recognized that this condition causes severe damage to the plasma membrane, especially in cells with high PUFA content such as spermatozoa [1,2]. Indeed, oxidative stress-induced radical oxygen species attack membrane phospholipid-bound PUFA leading to lipid peroxidation and inducing spermatozoa DNA and protein damage and was shown to impair spermatozoa properties and sperm function [25,26]. Accordingly, administration of antioxidants such as vitamins A, C, and E resulted in an improvement in spermatozoa motility and morphology in men with spermatic pathology [27].

CONCLUSION

While our study cannot establish causality, it seems that a hostile environment that alters anti-oxidants/pro-oxidants balance in sperm could affect the spermatozoa function. A high n-3 PUFA and antioxidants intake with a low intake in SAFA and MUFA, associated with a reduction of exposure to oxidant substances such as tobacco and toxics could contribute to improving semen characteristics and treat sperm abnormalities.

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Declaration of Interest:
The authors declare no conflict of interest.

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