Avicenna J Med Biochem. 2014 September; 2(1): e19607. DOI: 10.17795/ajmb-19607

Published online 2014 September 25. **Research Article**

Correlation Between Lipid Profile of Sperm Cells and Seminal Plasma With Lipid Profile of Serum in Infertile Men

Heidar Tavilani [1,*]; Akram Vatannejad [2]; Maryam Akbarzadeh [3]; Mojgan Atabakhash [4]; Saeed Khosropour [4]; Abozar Mohaghgeghi [4]

[1]Urology and Nephrology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran
[2]Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, IR Iran
[3]Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, IR Iran
[4]Student Research Committee, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Heidar Tavilani, Urology and Nephrology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-8138381590, Fax: +98-8138380130, E-mail: tavilani@gmail.com

Received: April 21, 2014; **Accepted:** June 2, 2014

Background: Lipids play an important role in the functional activity of sperm cells.
Objectives: The main goal of this study was to assess the correlation between the levels of cholesterol, phospholipids and triacylglycerols found in serum, with the lipid levels of semen in infertile men.
Patients and Methods: Cholesterol, phospholipids and triacylglycerols in sperm cells, seminal plasma and serum were assayed in 60 infertile men.
Results: There were no significant relationships between the concentration of sperm and seminal plasma cholesterol with serum cholesterol ($r = 0.003$, $P = 0.9$ and $r = 0.055$, $P = 0.67$, respectively), between the concentration of sperm and seminal plasma triglycerides with serum triglycerides ($r = 0.16$, $P = 0.2$ and $r = -0.039$, $P = 0.77$, respectively), or between the concentration of sperm and seminal plasma phospholipids with serum phospholipids ($r = 0.18$, $P = 0.16$ and $r = 0.053$, $P = 0.69$, respectively).
Conclusions: These results suggest that serum cholesterol, phospholipids and triacylglycerols have no effect on the levels of cholesterol, phospholipids and triacylglycerols of spermatozoa and seminal plasma. Our findings suggest that sperm lipid content is regulated locally within the male reproductive tract.

Keywords: Cholesterol; Lipids; Phospholipid; Serum; Semen Analysis; Spermatozoa

1. Background

Lipids have an important role in the functional activity of sperm cells (1, 2). Sperm viability, maturity, capacitation and fertilization are affected by lipid components (3). Phospholipids and cholesterol are important components of human plasma membranes and they are required for membrane permeability, fluidity, and capacitation (4). Changes in the lipid composition of spermatozoa have been reported in infertile males (5, 6). In addition to alterations in the lipid composition of human spermatozoa, there have also been reports on changes in the serum lipid profile of infertile men. Ramirez-Torres et al. reported a 65% incidence of hyperlipidemia, including hypercholesterolemia and triglyceridemia in 106 male partners of infertile couples (7). Ergun et al. demonstrated a significant correlation between plasma lipid concentrations and sperm motility, and reported that hypertriglyceridemia may have deleterious effects on spermatogenesis (8). In animal studies, researchers examined the effects of a high-cholesterol diet on male fertility and concluded that a high-cholesterol diet resulted in a significant decline in fertility and sperm characteristics, decrease in sperm acrosome reaction kinetics, and detrimental effects on Leydig and Sertoli cell secretory capacity (9, 10). Although these studies suggest a link between dyslipidemia and infertility, no clear mechanism was reported. The question of whether the lipid profile of serum can have an effect on the lipid profile of semen, has not been answered. Although the destructive effects of serum lipid alterations, as well as semen lipids changes on reproductive ability are understood, the correlation between serum lipids and semen lipid concentrations is still unknown. To the best of our knowledge, little information is available about the correlation between serum lipids and semen lipids.

2. Objectives

The main goal of this study was to assess the correlation between the serum levels of cholesterol, phospholipids and triacylglycerol with the semen lipids of infertile men.

3. Patients and Methods

The population of this cross-sectional study consisted of 60 Iranian men with defined infertility, and without any liver or renal disease, thyroid disorders, diabetes mellitus, or history of using anti-hyperlipidemic drugs, or any medication affecting lipid metabolism such as statins or diuretics. Semen samples of infertile males were collected by masturbation following 3 days of abstinence. After liquefaction, semen volume, sperm concentration, total sperm count, morphology, and motility grades (a: rapid progressive; b: slow progressive; c: non-progressive; and d: immotile) were determined using World Health Organization standard procedures (11). Written informed consent was obtained from all those enrolled, according to the criteria of the Ethical Committee of Hamadan University of Medical Sciences. Blood samples were collected after overnight fasting from 60 subjects, and after serum isolation; samples were stored at -20°C until analyses.

3.1. Sperm Fractionation

Aliquots of 1 mL of the liquefied semen were layered on top of the upper layer of 40% and 80% Pure Sperm gradient (Nidacon International, Sweden), then centrifuged at 400 × g for 20 minutes (12). The resulting interfaces at 40% and 80% (fraction 1), 80% and pellet (fraction 2), and pellet (fraction 3), were isolated and transferred to separate tubes (13). An aliquot of fraction 3 was used to evaluate sperm motility, morphology, and concentration. Suspensions of sperm from the different Pure Sperm fraction 3 were diluted in 2 mL phosphate buffer saline (PBS) and centrifuged at 800 g for 8 minutes; this washing was repeated. The pellet was then resuspended in 1 mL PBS and stored at -80°C (14). Sperm of fraction 3 were used for lipid extraction and determination of cholesterol, phospholipid, and triacylglycerol levels.

3.2. Extraction and Analysis of Lipids

Sperm cells of fraction 3 isolated by Pure Sperm gradient as well as seminal plasma were used for lipid extraction. Lipids of spermatozoa and seminal plasma were extracted with 6 volumes of chloroform-methanol (2/1, V/V), centrifuged at 800 × g for 3 minutes, and the resulting lower phase aspirated and dried under a stream of nitrogen (14). The cholesterol of the sperm cells and seminal plasma was assayed using Liebermann-Burchard reagent (15). The phospholipid level was determined using a modification of the method by Bartlett (16), and the triacylglycerol level of the sperm cells and seminal plasma was determined using acetyl acetone with the method of Gottfried and Rosenberg (17). Serum concentrations of cholesterol and triacylglycerols were measured by enzymatic methods (Pars Azmoon kits, Iran).

3.3. Statistical Analysis

Results were presented as mean ± SD. The correlation between the cholesterol, phospholipids and triglycerides of the serum, with the lipids of seminal plasma and sperm cells from fraction 3, was investigated using non-parametric a Spearman's coefficient (r). In addition, a non-parametric Spearman's coefficient was used to determine the correlation between serum lipids with sperm morphology and motility.

4. Results

The main semen parameters of the groups are illustrated in Table 1. The concentrations of cholesterol, phospholipids, and triacylglycerols in seminal plasma, sperm fraction 3 and serum are shown in Table 2. There were no significant relationships found; between the concentration of sperm and seminal plasma cholesterol with serum cholesterol (r = 0.003, P = 0.9 and r = 0.055, P = 0.67 respectively), between the concentration of sperm and seminal plasma triglyceride with serum triglyceride (r = 0.16, P = 0.2 and r = -0.039, P = 0.77, respectively), or between the concentration of sperm and

Table 1. Basic Parameters of Semen Sample in Total Subjects (n = 60) [a, b]

	Semen	Fraction 3
Motility grade a, %	3.5 ± 3	7.7 ± 6.9
Motility grade b, %	17.85 ± 9.7	28 ± 11.8
Motility grade c, %	21.3 ± 11.9	24.5 ± 13.8
Motility grade d, %	57.2 ± 18.50	39.7 ± 13.7
Morphology, %	16.4 ± 7.2	25.6 ± 10.7
Pathologic head, %	60.4 ± 11.2	64.3 ± 11.8
Pathologic mid-piece, %	13.1 ± 6.1	6.7 ± 4.9
Pathologic tail, %	10 ± 7.5	3.4 ± 4.6
Concentration, 10^6/mL	107.9 ± 49.6	28.8 ± 20.1

[a] Semen analysis was conducted on fresh ejaculate (semen) and after isolation by pureSpermgradiant (fraction 3).
[b] data are presented as mean ± SD

Table 2. Content (mean ± SD) of Cholesterol, Phospholipid and Triacylglycerol of Sperm Fraction 3, Seminal Plasma and Serum From Subjects (n=60).

	Cholesterol	Phospholipids	Triacylglycerols
Sperm fraction 3, nmol/10^6	0.63 ± 0.66	1.4 ± 0.95	0.19 ± 0.19
Seminal plasma,µmol/mL	1.45 ± 0.48	0.38 ± 0.23	5.40 ± 1.75
Serum, mmol/L	4.84 ± 1	1.2 ± 0.88	1.5 ± 0.71

seminal plasma phospholipids with serum phospholipids (r = 0.18, P = 0.16 and r = 0.053, P = 0.69, respectively). Generally, we did not observe any significant association between the levels of serum cholesterol, phospholipids and triglycerides with morphology and motility grades of sperm cells from the semen or fraction 3 (P > 0.05). However, the correlation between serum phospholipids with motility grade a from sperm fraction 3 (r = 0.27, P = 0.03) and serum triglycerides with motility grade b from sperm fraction 3 (r = 0.26, P = 0.03), were significant.

5. Discussion

The present study aimed to assess the correlation between serum lipid concentrations and variations in seminal lipid parameters in infertile men. We found no relationship between the concentration of cholesterol, phospholipids and triacylglycerols in serum, spermatozoa or seminal plasma of the infertile men under present examination, which is consistent with the findings of several other authors (18). Grizard et al. compared the effect of hypercholesterolemia and normocholesterolemia on the spermatozoa and seminal content of cholesterol and phospholipids. They suggested that hypercholesterolemia has no effect on cholesterol and phospholipid levels in spermatozoa and seminal plasma (18). Since cholesterol has a major role in the sperm membrane, which is essential for sperm cell function, it can be assumed that an increase of cholesterol level in the blood will also increase the cholesterol content of semen. This hypothesis was not confirmed in the presented study. There appears to be no correlation between the amount of cholesterol in the serum and in sperm or seminal plasma, suggesting that sperm cholesterol content is regulated locally within the male reproductive tract (4, 19, 20). For proper function of spermatozoa, cholesterol and phospholipids should be regulated accurately. In the male reproductive tract lipid homeostasis is done by testicular and post-testicular function (20, 21). Our results showed no correlation between serum lipids with sperm parameters, which is consistent with the findings of some other authors. Khalili et al. reported that the concentrations of serum lipids were not generally related to the quality of semen parameters (22). Nonetheless, our results are not consistent with the results of some other studies, in which animals were fed with a high-fat diet (9, 10). These results suggest that serum cholesterol, phospholipids and triacylglycerols have no effect on the levels of cholesterol, phospholipids and triacylglycerolsin spermatozoa and seminal plasma, and in addition, they do not cause any alteration of semen parameters. Our findings suggest that sperm lipidlevelsare regulated locally within the male reproductive tract.

Acknowledgements

This study was supported by Hamadan University of Medical Science.

Authors' Contributions

Heidar Tavilani: design of the study and critical revision; Saeed Khosropour and Abozar Mohaghgeghi: statistical analysis and interpretation of data; Akram Vatannejad, Maryam Akbarzadeh, and Mojgan Atabakhash: conducted the experiments.

Funding/Support

This study was supported by Hamadan University of Medical Sciences.

References

1. Sebastian SM, Selvaraj S, Aruldhas MM, Govindarajulu P. Pattern of neutral and phospholipids in the semen of normospermic, oligospermic and azoospermic men. *J Reprod Fertil.* 1987;**79**(2):373–8.
2. Tavilani H, Doosti M, Abdi K, Vaisiraygani A, Joshaghani HR. Decreased polyunsaturated and increased saturated fatty acid concentration in spermatozoa from asthenozoospermic males as compared with normozoospermic males. *Andrologia.* 2006;**38**(5):173–8.
3. Lenzi A, Picardo M, Gandini L, Dondero F. Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. *Hum Reprod Update.* 1996;**2**(3):246–56.
4. Travis AJ, Kopf GS. The role of cholesterol efflux in regulating the fertilization potential of mammalian spermatozoa. *J Clin Invest.* 2002;**110**(6):731–6.
5. Tavilani H, Doosti M, Nourmohammadi I, Mahjub H, Vaisiraygani A, Salimi S, et al. Lipid composition of spermatozoa in normozoospermic and asthenozoospermic males. *Prostaglandins Leukot Essent Fatty Acids.* 2007;**77**(1):45–50.
6. Tavilani H, Goodarzi MT, Doosti M, Vaisi-Raygani A, Hassanzadeh T, Salimi S, et al. Relationship between seminal antioxidant enzymes and the phospholipid and fatty acid composition of spermatozoa. *Reprod Biomed Online.* 2008;**16**(5):649–56.
7. Ramirez-Torres MA, Carrera A, Zambrana M. [High incidence of hyperestrogenemia and dyslipidemia in a group of infertile men]. *Ginecol Obstet Mex.* 2000;**68**:224–9.
8. Ergun A, Kose SK, Aydos K, Ata A, Avci A. Correlation of seminal parameters with serum lipid profile and sex hormones. *Arch Androl.* 2007;**53**(1):21–3.
9. Shalaby MA, el-Zorba HY, Kamel GM. Effect of alpha-tocopherol and simvastatin on male fertility in hypercholesterolemic rats. *Pharmacol Res.* 2004;**50**(2):137–42.
10. Saez Lancellotti TE, Boarelli PV, Monclus MA, Cabrillana ME, Clementi MA, Espinola LS, et al. Hypercholesterolemia impaired sperm functionality in rabbits. *PLoS One.* 2010;**5**(10): e13457.
11. World Health Organization.. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction.* 4th ed. Cambridge, UK: Cambridge University Press; 1999.
12. Allamaneni SS, Agarwal A, Rama S, Ranganathan P, Sharma RK. Comparative study on density gradients and swim-up preparation techniques utilizing neat and cryopreserved spermatozoa. *Asian J Androl.* 2005;**7**(1):86–92.
13. Setarehbadi R, Atabakhash M, Fattahi A, Mohagheghi A, Vaisi-Raygani A, Mahjub H, et al. Variation of cholesterol, phospholipid and triacylglycerol content in subsets of human spermatozoa isolated by density gradient. *Avicenna J Med Biochem.* 2013;**1**(1).
14. Calamera J, Buffone M, Ollero M, Alvarez J, Doncel GF. Superoxide dismutase content and fatty acid composition in subsets of human spermatozoa from normozoospermic, asthenozoospermic, and polyzoospermic semen samples. *Mol Reprod Dev.* 2003;**66**(4):422–30.
15. Kim E, Goldberg M. Serum cholesterol assay using a stable Liebermann-Burchard reagent. *Clin Chem.* 1969;**15**(12):1171–9.
16. Bartlett GR. Phosphorous assay in column chromatography. *J Biol Chem.* 1959;**234**:466–8.
17. Gottfried SP, Rosenberg B. Improved manual spectrophotomet-

ric procedure for determination of serum triglycerides. *Clin Chem.* 1973;**19**(9):1077–8.
18. Grizard G, Sion B, Jouanel P, Benoit P, Boucher D. Cholesterol, phospholipids and markers of the function of the accessory sex glands in the semen of men with hypercholesterolaemia. *Int J Androl.* 1995;**18**(3):151–6.
19. Saez F, Ouvrier A, Drevet JR. Epididymis cholesterol homeostasis and sperm fertilizing ability. *Asian J Androl.* 2011;**13**(1):11–7.
20. Maqdasy S, Baptissart M, Vega A, Baron S, Lobaccaro JM, Volle DH. Cholesterol and male fertility: what about orphans and adopted? *Mol Cell Endocrinol.* 2013;**368**(1-2):30–46.
21. Lobaccaro JMA, Brugnon F, Volle DH, Baron S. Lipid metabolism and infertility: is there a link? *Clin Lipidol.* 2012;**7**(5):485–8.
22. Khalili MA, Zare-Zadeh N, Hashemi H. Correlation between serum lipids profile with sperm parameters of infertile men with abnormal semen analysis. *Iran J Reprod Med.* 2009;**7**(3):123–7.