AviJMB
Avicenna Journal of Medical Biochemistry
journal homepage: http://AJMB.umsha.ac.ir

Original Article

Variation of cholesterol, phospholipid and triacylglycerol content in subsets of human spermatozoa isolated by density gradient

Rohollah Setarehbadi[1], Mojgan Atabakhash[1], Amir Fattahi[1], Aboozar Mohagheghi[1], Asad Vaisi-Raygani[2], Hossain Mahjub[3] and Heidar Tavilani[4]*

[1]*Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
[2]*Fertility and Infertility Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[3]*Department of Epidemiology and Biostatistics, Faculty of Public Health, Hamadan University of Medical Science, Hamadan, Iran*
[4]*Urology & Nephrology Research Center, Hamadan University of Medical Sciences, Hamadan Iran*

*** CORRESPONDENCE**
Heidar Tavilani
Tel: +98 8118380717
Fax: +98 8118380313
E-mail: tavilani@gmail.com

ARTICLE HISTORY
Received: 04 July 2013
Revised: 10 September 2013
Accepted: 20 September 2013
Available online: 28 September 2013

Citation:
Setarehbadi R, Atabakhash M, Fattahi A, Mohagheghi A, Vaisi-Raygani A, Mahjub H, Tavilani H. Variation of cholesterol, phospholipid and triacylglycerol content in subsets of human spermatozoa isolated by density gradient. Avi J Med Biochem. 2013; 1(1): 41-45.

ABSTRACT

Objectives: Lipid components of spermatozoa have an important role in its functional activity. In this study the concentration of cholesterol, phospholipid, triacylglycerol and total lipid in different subsets of human spermatozoa, isolated as three fractions by a discontinuous PureSperm gradient, was determined.

Methods: Aliquots of the liquefied semen samples (n=107) were layered on top of the upper layer of 40 and 80% PureSperm gradient. The resulting interfaces 40 and 80% (fraction 1), 80% and pellet (fraction 2), and pellet (fraction 3) aspirated and transferred into separate tubes. Lipids were extracted with 6 volumes of chloroform-methanol (2/1, V/V) and the concentration of cholesterol, phospholipid, triacylglycerol were determined by colorimetric method.

Results: Percent of sperm with normal morphology was significantly higher in sperm fraction 3 compared to fraction 2 and 1, while percent of sperm with midpiece and tail defect was significantly higher in fraction 1 compared to fractions 2 and 3 ($P<0.01$). The amount of cholesterol, phospholipid and total lipid of sperm fractions 2 and 3 were about 1.5-fold higher than that of found in sperm from fraction 1 ($P<0.05$).

Conclusion: This result suggests that the total lipid contents of sperms increase with increasing normal morphology from fraction 1 to 3.

Keywords: *Centrifugation, Density Gradient; Lipids; Spermatozoa*

Introduction

Lipid components of spermatozoa have an important role in its functional activity [1, 2]. Lipid plays an active role in sperm capacitation and fertilization. Davis has shown that content of cholesterol and phospholipid of spermatozoa has an essential role in capacitation process [3]. Tavilani et al. reported alteration in sperm phospholipid and fatty acid composition of asthenozoospermic males compared to normozoospermic individuals [4, 5]. In addition, lipid plays a central role in sperm maturation and change of spermatozoa lipid component is one of the important biochemical events which occur during this process. It has been suggested that lipids are essential for the viability and function of spermatozoa [6, 7].

Semen sample contains variable proportions of mature and immature spermatozoa and some individual spermatozoa may contain several-times higher or lower concentration of lipids compared to the others. Some studies reported the lipid content of spermatozoa which isolated by Percoll, whereas there is little information

about of the lipid concentration of spermatozoa which isolated by PureSperm gradient [2, 8]. Percoll [(polyvinylpyrrolidone (PVP)-coated silica] is toxic and is replaced by PureSperm [9]. Currently, PureSperm gradient is used for isolation of sperm cells in most infertility centers. Separation of sperm with the use of PureSperm gradient is a standard technique for isolation and preparation of sperm for use in assisted reproductive techniques [2, 9-11]. Since lipid composition of sperm has significant effect on viability, maturity and function of this cell [2], thus, it is essential to investigate the lipid content in different subsets of spermatozoa isolated by discontinuous PureSperm gradient.

In this study, the concentration of cholesterol, phospholipid, triacylglycerol and total lipid (sum of cholesterol, phospholipid, and triacylglycerol) was determined in subsets of human spermatozoa, isolated as three fractions by a discontinuous PureSperm gradient.

Materials and Methods

Semen specimens were obtained from 107 males attending the Fatemieh Fertility Clinic for infertility evaluation. Subjects had no diabetes or thyroid disease, and had no history of using lipid-lowering drugs. Written informed consent was obtained from all participants, according to the criteria of the Ethical Committee of Hamadan University of Medical Sciences (Hamadan, Iran). Semen samples were collected by masturbation following 3 days of abstinence. After liquefaction, semen volume, sperm concentration (haemocytometer), total sperm count, morphology (Papanicolaou staining method) and motility grades (a: rapid progressive; b: slow progressive; c: non-progressive; d: immotile) were determined according to the World Health Organization standard procedures [12].

PureSperm Fractionation

Aliquots of 1 ml of the liquefied semen were layered on top of the upper layer of 40 and 80% PureSperm gradients and centrifuged at 400×g for 20 min. The resulting interfaces 40 and 80% (fraction 1), 80% and pellet (fraction 2) and pellet (fraction 3) aspirated and transferred into separate tubes [13]. An aliquot of each fraction was used to evaluate sperm motility, morphology and concentration. Suspensions of sperm from different PureSperm fractions were diluted in 2 ml phosphate buffer saline (PBS) and centrifuged at 800×g for 8 min. After repeating washing process, pellet was resuspended in 1 ml PBS and stored at –80ºC [14].

Extraction and analysis of sperm lipids

Lipids were extracted by adding of 6 volumes of chloroform-methanol (2/1, V/V) to the samples and centrifugation at 800×g for 3 min. The resulting lower phase was then aspirated and dried under a stream of nitrogen [14].

Phospholipid content of sperms was assayed using spectrophotometric method of Bartlett [15], whereas the cholesterol level of sperms was determined using Liberman-Buchard reagent [16]. To determine triacylglycerol, Gottfried-Rosenberg method was carried out [17]. The results of phospholipid, cholesterol and triacylglycerol concentration were extrapolated to 10^6 spermatozoa.

Statistical Analysis

Results are expressed as mean ± SD. To assess the normality of the evaluated variables, Kolmogorov Smirnov test was performed. For comparing the means in different fractions ANOVA test was used. Also, Tukey's Post Hoc was used to compare pairwise groups. Statistical significance was assumed at the $P<0.05$ level.

Results

The semen profiles of subjects are given in Table 1 and sperm parameters of different fractions obtained by PureSperm fractionation are shown in Table 2 and 3. Percent of motility grade a, b, c were significantly higher in sperm of fraction 3 compared to sperm from fraction 2 and 1 ($P<0.01$). In contrast, percent of motility grade d was significantly higher in sperm fraction 1 compared to sperm from fractions 2 and 3 ($P<0.01$).

Table 1. Basic parameters of semen sample from studied subjects (n=107)

Semen parameters	mean ± SD
Volume (ml)	3.5 ± 1.16
Sperm concentration (10^6/ml)	108.5 ± 49
Sperm normal morphology (%)	16.3 ± 7.2
Sperm motility grade a[1] (%)	3.5 ± 3
Sperm motility grade b[1] (%)	17.9 ± 9.6
Sperm motility grade c[1] (%)	21.2 ± 11.8
Sperm motility grade d [1] (%)	57.2 ± 18.4

[1] Grade of sperm movement according to WHO criteria. a, rapid progressive; b, slow progressive; c, non progressive; d, immotile.

In addition, sperm concentration of sperm fraction 1 was significantly higher than from sperm fractions 2 and 3 ($P<0.05$). Percent of sperm with normal morphology was significantly higher in sperm fraction 3 compared to fraction 2 and 1 ($P<0.01$). Percent of sperm with midpiece and tail defect were significantly higher in fraction 1 compared to fractions 2 and 3 ($P<0.01$), although, a same percent of sperms with head defect was observed in all fractions.

The content of cholesterol, phospholipid, triacylglycerol and total lipid of different PureSperm fractions are illustrated in Table 4. There were significant difference between levels of cholesterol, phospholipid and triacylglycerol in fractions 1, 2 and 3 ($P<0.05$). The amounts of cholesterol, phospholipid and total lipid of sperm fractions 2 and 3 were about 1.5-fold higher than that of found in sperm from fraction 1 ($P<0.05$). However, content of triacylglycerol from sperm fraction 2 was significantly higher compared to sperm fraction 1 and 3 ($P<0.05$).

Table 2. Sperm motility grades and concentration from sperm fractions 1, 2 and 3 obtained by puresperm fractionation (n=107)

	Motility grade a[1] (%)	Motility grade b[1] (%)	Motility grade c[1] (%)	Motility grade d [1] (%)	Sperm concentration (10^6/ml)
Fraction1	1.9 ± 3.2	8.6 ± 7.5	14.8 ± 11.7	74.6 ± 16.2*	27.4 ± 16.8*
Fraction2	5.1 ± 6.5	18.4 ± 10.5	21.1 ± 13.7	55 ± 17.7	22.8 ± 14.7
Fraction3	7.7 ± 6.8*	28 ± 11.8*	24.5 ± 13.7*	39.6 ± 13.6	22.8 ± 20

[1]Grade of sperm movement according to WHO criteria. a, rapid progressive; b, slow progressive; c, nonprogressive; d, immotile.
*$P<0.05$ fraction 1 vs fraction 2 and 3.

Table 3. Sperm morphology from sperm fractions 1, 2 and 3 obtained by puresperm fractionations (n=107)

	Normal morphology (%)	Head defect (%)	Midpiece defect (%)	Tail defect (%)
Fraction1	11.1 ± 6.1	63.1 ± 11.5	13.3 ± 7**	12.3 ± 8.2**
Fraction2	19.2 ± 9.1	65.1 ± 10.5	9.3 ± 4.9	6.2 ± 5.9
Fraction3	25.6 ± 10.7*	64.2 ± 11.7	6.7 ± 4.9	3.4 ± 4

*$P<0.01$ fraction 3 vs faction 2 and 1
**$P<0.01$ fraction 1 vs fraction 2 and 3

Table 4. The content of cholesterol, phospholipid, triacylglycerol and total lipid (sum of cholesterol, phospholipid, triacylglycerol) of puresperm fraction 1, 2 and 3 (n=107)

	Cholesterol (nmol /10^6 sperms)	Phospholipid (nmol /10^6 sperms)	Triacylglycerol (nmol /10^6 sperms)	Total lipid (nmol /10^6 sperms)
Fraction1	0.47 ±0.36	0.84 ±0.54	0.21 ±0.15	1.5 ±0.15
Fraction2	0.63 ±0.53	1.29 ±0.76	0.27 ±0.24**	2.18±1.35
Fraction3	0.63 ±0.65*	1.36 ±0.95*	0.19±0.19	2.16 ±1.6*

*$P<0.05$ fraction3 and 2 vs fraction1
**$P<0.05$ fraction 2 vs fraction 1 and 3

Discussion

In the present study, the concentration of cholesterol, phospholipid, triacylglycerol and total lipid in subsets of human spermatozoa, isolated as three fractions by a discontinuous PureSperm gradient, was determined. Our results showed sperm parameters including motility grade a, b and normal morphology were significantly higher in sperm of fraction 3 compared with fraction 2 and 1. Furthermore, percent of sperm with midpiece defect was significantly higher in fraction 1 compared with fractions 2 and 3. In the other words, from fraction 1 to fraction 3 degree of sperm maturation increased which is in agreement with the results of other studies [10, 18- 20]

Our research showed that fraction 2 and 3 had the same phospholipid, cholesterol, and total lipid contents as nmol/10^6 sperm, while fraction 3 had slightly lower triacylglycerol content. Present study also showed that total lipid content as well as phospholipid and cholesterol was lower in fraction 1 compared with fraction 2 and 3. Lower total lipid content of sperm from fraction1, which result in lower density of sperm, can be partly responsible for isolation of sperm cells in

fraction 1. Indeed, fraction 1 has the lowest density compared to fraction 2 and 3 and it is reasonable that sperms with lower density are collected in this fraction. However we did not exclude the role of other components of sperm in determination of density in these cells. Our results suggested that the phospholipid and cholesterol content of fraction 2 and 3 are those for the membrane composition of mature sperm that have undergone shedding of the residual body in an optimal manner. Since sperm with residual body are categorized in the midpiece defect, this opinion is reinforced by our finding which showed percent of sperm with midpiece defect was higher in fraction 1 than fraction 2 and 3. Other studies showed that PureSperm gradients significantly improved the percentage of mature sperm and decreased percentage of sperm with midpiece and residual bodies defect when compared with those of the original semen samples [18]. This result suggested that total lipid contents of sperms increase with increasing normal morphology from fraction 1 to 3.

In this study, we found that sperm fractions 2 and 3 had equal content of cholesterol and phospholipid, but the content of triacylglycerol in these two fractions was different. In the other words, it is possible that isolation of sperm cells in the fraction 2 and 3 may result from difference in triacylglycerol content of these cells. The exact role of triacylglycerol in sperm cells has not been elucidated well, whereas the roles of phospholipid and cholesterol have been discussed in details. The role of spermatozoa membrane lipids, such as cholesterol and phospholipids have been the focus of investigation in most studies [2, 21]. One reason for paying no attention to triacylglycerol of sperm, may be because of the fact that triacylglycerol is not in a membrane compartment.

Conclusion

Our findings showed that there are variability in content of cholesterol, phospholipid and triacylglycerol in sperm cells fractionated by discontinuous PureSperm gradient.

Acknowledgments

The authors are grateful to Professor Bayard T. Storey, University of Pennsylvania, for review of this manuscript. This research was supported by Hamadan University of Medical Sciences.

Conflict of interest

The authors report no conflict of interest.

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; 79:373–8.
2. Zalata A, Hassan A, Christophe A, Comhaire F, Mostafa T. Cholesterol and desmosterol in two sperm populations separated on Sil-Select gradient. Int J Androl 2010; 33:528-35.
3. Davis BK. Timing of fertilization in mammals: sperm cholesterol/phospholipid ratio as a determinant of the capacitation interval. Proc Natl Acad Sci USA 1981; 78:7560-4.
4. Tavilani H, Doosti M, Abdi K, Vaisiraygan A, Joshaghani HR. Decreased polyunsaturated and increased saturated fatty acid concentration in spermatozoa from asthenozoospermic males as compared with normozoospermic males. Andrologia 2006; 38:173-8.
5. Tavilan H, Goodarzi MT, Doosti M, Vaisi-Raygani A, Hassanzadeh T, Salimi S, Joshaghani HR. Relationship between seminal antioxidant enzymes and the phospholipid and fatty acid composition of spermatozoa. Reprod Biomed Online 2008; 16:649-56.
6. 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; 2:246-56.
7. Zalata AA, Christophe AB, Depuydt CE, Schoonjans F, Comhaire FH. The fatty acid composition of phospholipids of spermatozoa from infertile patients. Mol Hum Reprod 1998; 4:111-8.
8. Sugkraroek P, Kates M, Leader A, Tanphaichitr N. Levels of cholesterol and phospholipids in freshly ejaculated sperm and Percoll-gradient-pelletted sperm from fertile and unexplained infertile men. Ferti Steril 1991; 55:820-7.
9. Fong CY, Peh G, Subramanian A, Gauthaman K, Bongso A. The use of discontinuous density gradients in stem cell research and application. Stem Cell Rev 2009; 5:428-34.
10. Centola GM, Herko R, Andolina E, Weisensel S. Comparison of sperm separation methods: effect on recovery, motility, motion parameters, and hyperactivation. Fertil Steril 1998; 70:1173-5.
11. Söderlund B, Lundin K. The use of silane-coated silica particles for density gradient centrifugation

in in-vitro fertilization. Hum Reprod 2000; 15:857-60.

12. World Health Organization WHO. Laboratory Manual for the Examination of Human Semen and Semen-cervical Mucus Interaction, 4th ed. Cambridge, UK7 Cambridge University Press; 1999. p. 4 – 23.
13. 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; 7:86-92.
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; 66:422-30.
15. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem 1959; 234:466-8.
16. Sonnenwirth AC, Jarett L. Gradwohls clinical laboratory methods and diagnosis, Mosby company, London; 1980. p. 280.
17. Gottfried SP, Rosenberg B. Improved manual spectrophotometric procedure for determination of serum triglycerides. Clin Chem 1973; 19:1077-8.
18. Chen MJ, Bongso A. Comparative evaluation of two density gradient preparations for sperm separation for medically assisted conception. Hum Reprod 1999; 14:759-64.
19. Claassens OE, Menkveld R, Harrison KL. Evaluation of three substitutes for Percoll in sperm isolation by density gradient centrifugation. Hum Reprod 1998; 13:3139-43.
20. Tomlinson MJ, Moffatt O, Manicardi GC, Bizzaro D, Afnan M, Sakkas D. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod 2001; 16:2160-5.
21. Tavilani H, Doosti M, Nourmohammadi I, et al. Lipid composition of spermatozoa in normozoospermic and asthenozoospermic males. Prostaglandins Leukot Essent Fatty Acids 2007; 7:45-50.