Iranian Biomedical Journal 16 (3): 162-168 (July 2012)
DOI: 10.6091/ibj.1081.2012

Fatty Acid Composition of Human Follicular Fluid Phospholipids and Fertilization Rate in Assisted Reproductive Techniques

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Received 24 April 2012; revised 23 May 2012; accepted 25 May 2012

ABSTRACT

Background: Fatty acids are known to be critically important in multiple biological functions. Phospholipid fatty acids of follicular fluid, an important microenvironment for the development of oocytes, may contribute to the women's fertility and the efficacy of assisted reproduction techniques. The aim of this study was to investigate the effect of fatty acid composition of follicular fluid phospholipids on women undergoing assisted reproductive techniques. **Methods:** Follicular fluid samples were obtained from 100 patients, referred to Tabriz Alzahra Hospital. Seventy-nine subjects underwent *in vitro* fertilization (IVF) and the remaining 21 underwent intracytoplasmic sperm injection (ICSI). Total lipid of follicular fluid was extracted and fatty acids were analyzed by gas-liquid chromatography. **Results:** Saturated fatty acids (SFA, $P = 0.002$) and the ratio of SFA to polyunsaturated fatty acids ($P = 0.001$) were correlated negatively with a number of mature oocytes after age adjustment. Linoleic acid ($P = 0.006$) was positively correlated, while the level of arachidonic acid was negatively correlated with fertility percentage after adjustment for body mass index, sperm count, sperm motility. **Conclusion:** Since phospholipids are one of the major components of lipid metabolism, the results of this study highlight the importance of this component in follicular fluid lipid metabolism. Consequently, it is proposed as an index in determination of the rate of success in assisted reproductive techniques such as IVF/ICSI. *Iran. Biomed. J. 16 (3): 162-168, 2012*

Keywords: Follicular fluid, Fatty acids, Assisted reproductive techniques

INTRODUCTION

Fertility is the result of direct interaction of sperm and oocyte, adhesion of the two cell membranes and the integration of male and female gamete genomes [1]. Infertility is a multifactorial disorder and life style has an important role in its appearance [2]. Assisted reproductive techniques *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) are used to treat a range of infertilities. However, these techniques are not always successful and factors leading to a successful IVF/ICSI are being extensively investigated [3]. Follicular fluid is a liquid that exists in women's ovary. This fluid plays an important role in oocyte development, women fertility and embryonic development [4]. Therefore, follicular fluid is often used as a medium supplement in IVF process [5, 6].

The proportions of specific fatty acids in blood or tissue are known to be useful risk markers for a variety of diseases [7]. The current evidence shows that some types of fatty acids are effective in the treatment and management of several diseases, which makes the evaluation of fatty acid status even more important. The fatty acid composition of follicular fluid phospholipids partially reflects that of the diet [8] and appears to be an important factor that affects their metabolism and could modulate fertility [9]. Fouladi-Nashta *et al.* [10] have shown the beneficial effects of increased level of dietary fat on the developmental potential of oocytes. These effects have been associated with changes in either fatty acid content of cell membrane phospholipids or cytoplasm of oocytes, which affect their developmental competence. According to Abayasekara and Wathes [11], it has

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been shown that alteration in dietary polyunsaturated fatty acids (PUFA) can change the content of PUFA in cell membrane and prostaglandin synthesis that affect the fertility. On the other hand, Mattos *et al.* [12] have found that prostaglandins, biologically active derivatives of PUFA, are important mediators in the ovulation. The proportions of saturated fatty acids (SFA) in oocytes and granulose cells have been found to be greater in summer and the percentages of monounsaturated fatty acids (MUFA) and PUFA in oocytes and granulosa cells found to be greater during the winter [13]. These changes have been correlated with dietary fatty acids and also low fertility of dairy cows during the summer [14].

We have recently shown that the level of cholesteryl ester transfer protein, a glycoprotein that mediates the neutral lipid transfer in follicular fluid, was correlated positively with the maturity and the percentage of oocyte fertilization [15]. These findings are consistent with the hypothesis that lipid content of follicular fluid influences its biological properties and consequently its efficacy in IVF/ICSI. Despite the evidences demonstrating the potential of fatty acids in human fertility, there are no direct results showing relationship between follicular fluid fatty acid composition and reproductive medicine. In the present study by employing gas-liquid chromatography technique, the correlation of follicular fluid fatty acid composition with fertility status was investigated in IVF/ICSI patients.

MATERIALS AND METHODS

Patients. A total of 100 patients were selected and referred to Tabriz Alzahra Hospital (Tabriz, Iran) in 2007-2009. All participants received a screening history, physical examination, measurements of serum hormone levels and blood counts, as previously reported by us [15]. Seventy-nine patients underwent IVF and the remaining 21 underwent ICSI. The subjects' body mass index (BMI) was calculated as measured weight divided by the square of measured height (kg/m^2). The mean age of subjects was 31.7 ± 5.46 years with no evidence of any disease. Healthy husband with no smoking habit were defined as including criteria. Uterus abnormalities, positive history of endocrine disease and inflammatory disorders such as thyroid and adrenal disorders, immune system defect and sexual hormones disorders were considered as exclusion criteria in this study.

Oocyte retrieval. Ovarian stimulation was achieved with a gonadotropin-releasing hormone agonist (Sereno, Switzerland)/follicle-stimulating hormone (FSH)-long down-regulation protocol [15]. Daily subcutaneous injection of 0.1 mg gonadotropin-releasing hormone analog was started at the 21th day of cycle. Controlled ovarian stimulation was started with recombinant human rFSH (Sereno, Switzerland) at the third day of menstrual cycle. The daily rFSH dose ranged between 150 and 300 IU, depending on BMI, age of the women, and the anticipated ovarian response. Dose adjustment was carried out according to follicular development and serum estradiol levels. Intramuscular hCG (1,000 IU, Choriomon, Meizler, Brazil) was administered when sonography revealed the average diameter of 3 preovulatory follicles had approached 18-20 mm. Oocyte retrieval was performed 36 h after hCG administration by vaginal ultrasound-guided puncture of the ovarian follicles. The collected oocytes were incubated with 5% CO_2 at 37ºC for 3-4 hours, and then were used for IVF and ICSI. Follicular fluid was collected in a sterile tube, centrifuged at 500 ×g for 5 min and kept frozen at -70°C until analysis. Oocytes with sporadic cumulus oophorus and zona pellucida and also a clear ooplasm were selected for insemination. The swim-up was used to prepare sperm for IVF [16]. Oocytes were inseminated with 250,000 sperm per oocytes in the IVF technique. In the ICSI group, a single motile sperm was injected into oocytes. After 24 h, oocytes were separated from surrounding granulosa cells. A maximum of three embryos were transferred at 4-8 cell stages after 48 h under ultrasound guidance. Clinical pregnancy was assessed by β-hCG test, 14 days after embryo transfer. Proportion of the oocytes with 2 pronuclei was estimated as fertilization rate. This project was approved by the Ethics Committee of Tabriz University of Medical Sciences. Before sampling, a written consent was received from the studied subjects.

Laboratory measurements. Fatty acid extraction from follicular fluid phospholipids was performed in three steps. At the first step total lipids were extracted from follicular fluid by Bligh and Dyer method [17]. At the second step, phospholipids were separated by thin layer chromatography using a silica gel plate and an organic solvent containing hexan/diethylether /glacial acetic acid (70:30:1). Phospholipids in this solvent system remained at the origin. At the third step, phospholipid fraction was scraped into glass tubes and extracted with chloroform:methanol solution. Then, fatty acids from phospholipids were derived by a direct transesterification method and analyzed by gas-liquid chromatography using a Buck Scientific model 610 gas chromatograph [18]. Fatty acid methyl ester derivatives formed from the isolated phospholipid fraction were separated on a TR-CN100 capillary column (60 × 0.25 mm). Tridecanoic acid (13:0) was used as an internal standard.

Table 1. Patients' clinical characteristics, biochemical profile of follicular fluid and semen parameters of husbands.

Age (years)	31.71 ± 5.46 (21-30)
Body mass index (kg/m^2)	25.52 ± 3.13 (20-34)
IVF parameters	
Mature oocytes	8.57 ± 4.19 (1-19)
Fertilized oocytes	5.00 ± 3.00 (0-13)
Pregnancy (%)	24
Semen characteristics	
Sperm count (10^6/ml)	59.51 ± 30.34 (28-120)
Sperm mobility (%)	66.17 ± 18.40 (10-90)

Values are expressed as mean ± SD or percent (ranges in parenthesis)

Statistical analysis. Values are presented as mean ± SD. The one way ANOVA with Tukey's post hoc pairwise comparison and multivariate analysis of variance and student's *t*-test were used for comparing means and ratios in different groups. *P* values of <0.05 were considered statistically significant. Analysis was carried out using SPSS 11.5 statistical software.

RESULTS

General characteristics, husband spermogram and follicular fluid parameters have been shown in Table 1.The average number of mature oocytes and of fertilized oocytes were about 8.57 ± 4.19 and 5.0 ± 3.0, respectively. The percentage of pregnancy in the studied population was 24%. The age of patients was inversely associated with the number of mature oocytes (r = - 0.21, *P* = 0.04).

Ten types of fatty acids were identified and quantified. Palmitic acid (16:0) was the major phospholipid fatty acid in the follicular fluid (Fig. 1). To investigate the correlation of fatty acids and phospholipids in follicular fluid and the number of mature oocytes in IVF and ICSI groups, patients were classified into different groups according to the index of mature oocytes (IMO), based on the number of mature oocytes at retrieval (IMO-I, >5; IMO-II, 6-9 and IMO-III, <10). Mean amounts of stearic acid (*P* = 0.03), SFA (*P* = 0.001) and the ratio of SFA to unsaturated PUFA (*P* = 0.002) were related negatively and arachidonic acid (*P* = 0.04) and PUFA (*P* = 0.007) were correlated positively with mature oocyte number after adjustment for age (Table 2).

Correlation between fertilization rate and fatty acid profile in follicular fluid phospholipids in IVF group was analyzed according to Table 3. Patients were classified into groups according to the index of fertility (IFR) based on the percentage of fertilized ratio oocytes at retrieval (IFR-I, >50%; IFR-II, 51-69% and IFR-III, <70%). The mean amounts of linoleic acid (*P* = 0.006) and arachidonic acid (*P* = 0.049) were different in the studied groups so that linoleic acid relation to FR was positive and arachidonic acid was negative, which was strengthened after age adjustment.

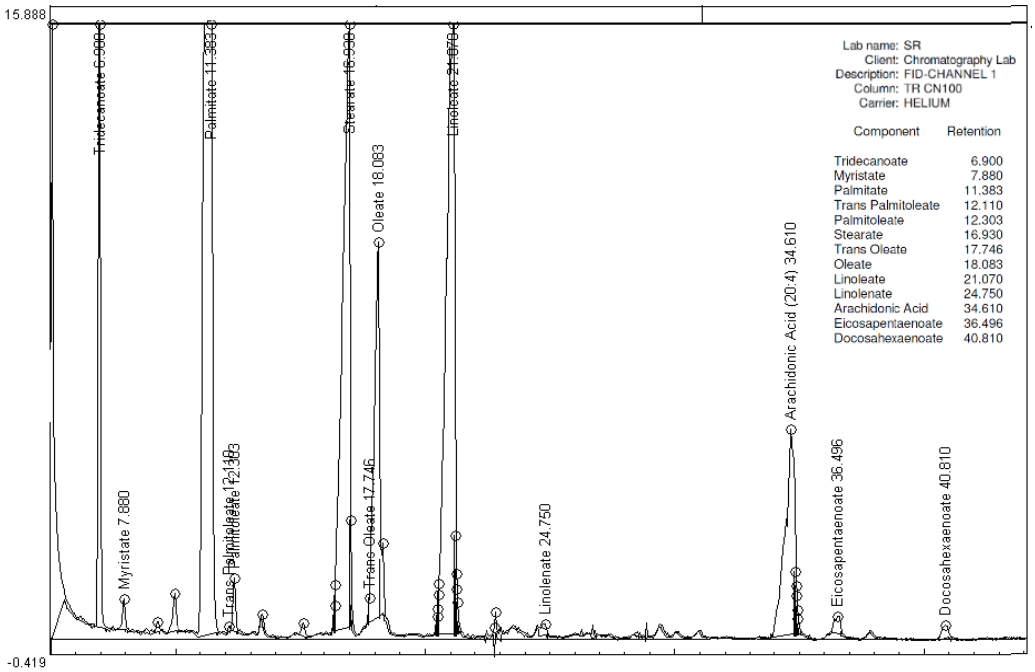

Fig. 1. Capillary gas chromatogram of fatty acid methyl esters of phospholipids isolated from follicular fluid. Fatty acid methyl ester derivatives formed from the isolated phospholipid fraction were separated on a TR-CN100 capillary column (60 × 0.25 mm). The fatty acids are well resolved. The analysis was completed in 50 minutes.

Table 2. Fatty acid composition of follicular fluid phospholipids in patients undergoing IVF/ICS with different number of mature oocytes.

IMO	IMO-I (n = 35)	IMO-II (n = 28)	IMO-III (n = 37)	*P*	*P**
16:0 (palmitic acid)	28.35 ± 1.77[a]	27.76 ± 1.22[a,b]	27.45 ± 1.34[b]	0.042	0.09
16:1 (palmitoleic acid)	2.17 ± 0.72	2.24 ± 0.68	2.27 ± 0.60	0.84	0.77
18:0 (stearic acid)	11.43 ± 1.41[a]	10.81 ± 1.54[b]	10.41 ± 1.21[b]	0.01	0.03
18:1tn-9 (trans oleic acid)	1.39 ± 0.58	1.62 ± 0.87	1.53 ± 0.79	0.47	0.48
18:1n-9 (oleic acid)	15.94 ± 1.22	15.75 ± 3.24	16.52 ± 1.53	0.24	0.35
18:2n-6 (linoleic acid)	20.32 ± 1.65	20.34 ± 1.60	20.82 ± 1.57	0.31	0.45
18:3n-6 (linolenic acid)	0.33 ± 0.72	0.37 ± 0.17	0.38 ± 0.20	0.40	0.65
20:4n-6 (arachidonic acid)	5.78 ± 0.99[a]	6.08 ± 0.79[a,b]	6.29 ± 0.99[c]	0.09	0.04
20:5n-3 (eicosapentaenoic acid)	0.24 ± 0.09	0.26 ± 0.11	0.26 ± 0.11	0.66	0.89
22:6n-3 (docosahexaenoic acid)	0.35 ± 0.12	0.34 ± 0.14	0.39 ± 0.13	0.13	0.24
SFA	39.77 ± 2.22[a]	38.64 ± 1.92[b]	37.83 ± 1.77[c]	<0.01	<0.01
MUFA	18.12 ± 1.34	17.97 ± 3.12	18.82 ± 1.65	0.20	0.31
PUFA	26.97 ± 1.46[a]	27.48 ± 1.33[a,b]	28.12 ± 1.67[b]	0.01	<0.01
n-3 fatty acids	0.92 ± 0.23	0.98 ± 0.21	1.05 ± 0.28	0.09	0.27
SFA/PUFA	1.48 ± 0.14[a]	1.41 ± 0.11[b]	1.35 ± 0.11[c]	<0.01	<0.01
n-6: n-3	30.73 ± 9.52	28.49 ± 7.54	27.82 ± 8.15	0.34	0.63

Values are % of total fatty acids, expressed as mean ± SD. ANOVA and Tukey's tests were used. [a, b, c] Values with different superscripts on the same row are significantly different. IMO (index of mature oocytes; number of mature oocytes at retrieval) -I, < 5; -II, 6-9; -III, > 10. SFA/PUFA, saturated to polyunsaturated fatty acids. *P** adjusted for age. MUFA, monounsaturated fatty acids

We also compared the mean amounts of fatty acids in follicular fluid phospholipids between the two pregnant and non-pregnant groups (Table 4). Mean amounts of linolenic acid ($P = 0.01$) and n-6 fatty acids ($P = 0.01$) showed significant differences between groups with higher amounts in pregnant group. The ratio of n-6/n-3 ($P = 0.02$) also indicated a significant difference between groups with a higher amount in non-pregnant group.

DISCUSSION

Infertility is a multifactorial disorder and every malfunction in sperm and oocyte generation and development process will result in this phenomenon. This study was carried out to investigate the effective factors on women fertility rates and its aim was to study the relation between fatty acid composition of follicular fluid phospholipids with IVF/ICSI successful rate in different laboratory and clinical levels.

Table 3. Fatty acid composition of follicular fluid phospholipids in patients undergoing IVF with different fertilization rates.

IFR	IFR-I (n = 24)	IFR-II (n = 26)	IFR-III (n = 24)	*P*	*P**
16:0 (palmitic acid)	27.92 ±1.74	27.85 ±1.65	27.97 ±1.62	0.93	0.88
16:1 (palmitoleic acid)	2.36 ± 0.65	2.17 ± 0.61	2.01 ± 0.54	0.12	0.13
18:0 (stearic acid)	11.22 ± 1.84	10.55 ± 1.16	11.17 ± 1.02	0.14	0.15
18:1tn-9 (trans oleic acid)	1.65 ± 0.64	1.58 ± 0.82	1.21 ± 0.75	0.09	0.11
18:1n-9 (oleic acid)	16.02 ± 1.84	16.10 ± 1.65	16.23 ± 1.14	0.84	0.92
18:2n-6 (linoleic acid)	19.92 ± 1.52[a]	20.82 ± 1.40[b]	21.21 ±1.54[c]	0.01	<0.01
18:3n-6 (linolenic acid)	0.38 ± 0.21	0.34 ± 0.19	0.37 ± 0.20	0.81	0.69
20:4n-6 (arachidonic acid)	6.13 ± 0.60[a]	6.06 ± 0.76[a,b]	5.85 ± 0.60[b]	0.30	0.05
20:5n-3 (eicosapentaenoic acid)	0.26 ± 0.12	0.23 ± 0.11	0.28 ± 0.11	0.24	0.13
22:6n-3 (docosahexaenoic acid)	0.36 ± 0.11	0.37 ± 0.14	0.32 ± 0.14	0.41	0.42
SFA	39.12 ± 2.72	38.24 ± 1.94	39.05 ± 1.72	0.30	0.67
MUFA	18.34 ± 1.82	18.37 ± 1.62	18.31 ± 1.24	0.97	0.81
PUFA	27.12 ± 1.40	27.82 ± 1.21	28.12 ± 1.58	0.01	0.13
n-3 fatty acids	0.98 ± 0.26	0.94 ± 0.27	1.01 ± 0.21	0.6	0.21
SFA/PUFA	1.45 ± 0.16	1.38 ± 0.11	1.39 ± 0.11	0.12	0.54
n-6: n-3	28.22 ± 7.94	31.52 ± 7.51	27.85 ± 8.12	0.37	0.21

Values are % of total fatty acids, expressed as mean ± SD. ANOVA and Tukey's tests were used. [a, b] Values with different superscripts on the same row are significantly different. IFR (index of fertilization rate, number of fertilized oocytes/number of mature oocytes × 100) –I, < 50%; -II, 51-69%; -III, > 70%. SFA/PUFA, saturated to polyunsaturated fatty acids. *P** adjusted for age. MUFA, monounsaturated fatty acids

Table 4. Fatty acid composition of follicular fluid phospholipids in pregnant and non-pregnant patients undergoing assisted reproductive techniques.

Pregnancy	Non-pregnant (n = 76)	Pregnant (n = 24)	*P*
16:0 (palmitic acid)	27.92 ± 1.64	27.82 ± 1.52	0.96
16:1 (palmitoleic acid)	2.34 ± 0.66	2.20 ± 0.69	0.84
18:0 (stearic acid)	10.82 ± 1.54	10.97 ± 1.15	0.77
18:1tn-9 (trans oleic acid)	1.56 ± 0.78	1.33 ± 0.63	0.18
18:1n-9 (oleic acid)	16.02 ± 2.23	16.24 ± 1.62	0.71
18:2n-6 (linoleic acid)	20.42 ± 1.57	20.6 ± 1.89	0.56
18:3n-6 (linolenic acid)	0.33 ± 0.16	0.46 ± 0.19	0.01
20:4n-6 (arachidonic acid)	6.15 ± 0.97	5.78 ± 0.86	0.10
20:5n-3 (eicosapentaenoic acid)	0.25 ± 0.10	0.26 ± 0.11	0.76
22:6n-3 (docosahexaenoic acid)	0.36 ± 0.13	0.37 ± 0.14	0.80
SFA	38.72 ± 2.34	38.70 ± 1.96	0.87
MUFA	18.22 ± 2.32	18.41 ± 1.52	0.76
PUFA	27.52 ± 1.45	27.5 ± 1.80	0.99
n-3 fatty acids	0.94 ± 0.24	1.05 ± 0.23	0.01
SFA/PUFA	1.41 ± 0.13	1.42 ± 0.14	0.86
n-6: n-3	30.24 ± 8.43	25.56 ± 8.02	0.02

Values are % of total fatty acids, expressed as mean ± SD. *t*-test was used. Biochemical pregnancy was assessed by β-hCG test 14 days after embryo transfer. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

According to our results in this study, which was in agreement with previous studies [19], the age of individuals had an inverse correlation with number of mature oocytes. Patients with infertility problems are likely to require long-term follow up. Regarding this fact, these individuals are generally over the age of 30 [19]. The selected population with the average age of 31 was younger in this study than that in the previous study [19]. Therefore, the age effect probably would be less on the intended factors.

In this study, the ratio of fertilized oocytes to mature oocytes and also pregnancy occurrence in studied population was lower compared to previous studies on the population of other countries [6, 19]. As we discussed previously [20], these could be attributed to higher negative effects of environmental factors, such as nutrition and life style in the studied population.

In previous studies, the fatty acid content of oocytes has been implicated in oocyte growth and differentiation [10, 21]. Since fatty acid composition of follicular fluid phospholipids is influenced by nutrition and individual metabolic condition, it is counted as an important index for the study of the effects of fatty acids on the rate of fertility. On the other hand, the oocyte membrane fluidity is dependent on the fatty acid composition of phospholipids, which can influence the quality of sperm acrosome reaction [22].

According to our results, SFA-like palmitic acid and stearic acid had negative correlation with the number of mature oocytes. In the studies carried out on domestic animals such as dairy cattle, it has been proven that the change in fatty acid composition of follicular fluid has a correlation with the maturity of the oocytes. In Zeron *et al.* [13] studies, it was shown that fatty acid composition of phospholipids undergoes seasonal changes so that the ratio of SFA is higher in summer whereas MUFA and PUFA have a higher percentage in follicular fluid and granulosa cells during winter. The reduction of PUFA in summer was related with that of embryo growth and the fertility in dairy cattle. In another study, it became clear that ewes treated with fish oil, a supplement rich in omega-3 fatty acid, had more follicles and oocytes, better quality oocytes, improved integrity of oocyte membranes, and an increased proportion of long-chain unsaturated fatty acids in plasma and cumulus cells [13]. The findings of our survey suggest that increase in polyunsaturated fatty acids to saturated ones in human follicular fluid phospholipids improves the maturity of oocytes and these oocytes have a higher efficiency, size and cell membrane integrity. This fact is probably because of the effects of the fatty acid composition on phospholipid metabolism. It has been shown that palmitic and stearic acid had inhibitory effects on the amount of fat stored in lipid droplets and a concomitant detrimental effect on oocyte developmental competence. Oleic acid, in contrast, had the opposite effect, causing an increase of lipid storage in lipid droplets and an improvement of oocyte developmental competence [23]. Consistent with our present findings, these results also suggested that the ratio of SFA and polyunsaturated fatty acid is relevant for maturity of oocytes. Oocytes are capable of incorporating fatty acids into their phospholipids; for metabolizing them

or for using them in cellular membranes. Therefore, it is possible that the differential fatty acid exposure of oocytes through follicular fluid directly influences the oocyte and embryo quality and thus the fertilization rates [23].

Among the studied fatty acids, the amount of linoleic acid had the most effect on fertilization rate. This fatty acid is the precursor for synthesis of many active fatty acid metabolites such as prostaglandins [24]. On the other hand, the amount of arachidonic acid had an inverse correlation with fertilization rate. Since this fatty acid is used in the production of inflammatory mediators [25], it seems that its increase in the phospholipids content has a negative effect on fertility. This fatty acid had a positive correlation with the number of mature oocytes, which has potential benefits. Higher concentrations of linoleic acid and lower concentrations of arachidonic acid in dominant follicles have been reported [9]. These findings are in agreement with the fact that the oocyte maturation process and its fertility capability are independent from each other to some extent and maybe influenced by different factors [26].

According to the obtained results of our study, n-3 fatty acids unlike n-6 fatty acids had a positive correlation with the pregnancy. Since n-3 fatty acids are classified as essential fatty acid [27]. Therefore, their amounts in follicular fluid are mostly affected by diet. On the other hand, it seems that the zygote attachment to the uterus wall may also be affected by the fatty acid composition of zygote membrane. Overall, these findings indicate that fatty acids may play distinct roles at different stages of oocyte maturation and early embryo development.

Present study indicates that phospholipid fatty acid profile of follicular fluid has a very important role in the oocyte maturity process, fertilization rate and the success rate of IVF/ICSI. Since phospholipids are one of the major components of lipid metabolism, the results of this study highlight the importance of this component in follicular fluid lipid metabolism and it is consequently proposed as an index in determination of the rate of success in assisted reproductive techniques such as IVF/ICSI.

ACKNOWLEDGMENTS

This study was partially supported by grants from the Research Center for Pharmaceutical Nanotechnology (RCPN, grant number 89), Tabriz University of Medical Sciences.

REFERENCES

1. Wassarman PM, Jovine L, Litscher ES, Qi H, Williams Z. Egg-sperm interactions at fertilization in mammals. *Eur J Obstet Gynecol Reprod Biol.2004 Jul;115 Suppl1:S57-60.*
2. Sharpe RM, Franks S. Environment, lifestyle and infertility--an inter-generational issue. *Nat Cell Biol.2002 Oct; 4 Suppl:s33-40.*
3. Ozmen B, Koutlaki N, Youssry M, Diedrich K, Al-Hasani S. DNA damage of human spermatozoa in assisted reproduction: origins, diagnosis, impacts and safety. *Reprod Biomed Online.2007 Mar;14(3):384-95*.
4. Revelli A, Delle PL, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod Biol Endocrinol. 2009 May;7:40.*
5. Tokumura A, Miyake M, Nishioka Y, Yamano S, Aono T, Fukuzawa K. Production of lysophosphatidic acids by lysophospholipase D in human follicular fluids of *in vitro* fertilization patients. *Biol Reprod.1999 Jul;61(1):195-9*.
6. Chi HJ, Kim DH, Koo JJ, Chang SS. The suitability and efficiency of human follicular fluid as a protein supplement in human *in vitro* fertilization programs. *Fertil Steril.1998 Nov;70(5):871-7*.
7. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood).2008 Jun;233(6):674-88;Epub 2008 Apr 11*.
8. Fouladi-Nashta AA, Wonnacott KE, Gutierrez CG, Gong JG, Sinclair KD, Garnsworthy PC et al. Oocyte quality in lactating dairy cows fed on high levels of n-3 and n-6 fatty acids. *Reproduction.2009 Nov;138(5):771-81;Epub 2009 Jul 24*.
9. Bender K, Walsh S, Evans AC, Fair T, Brennan L. Metabolite concentrations in follicular fluid may explain differences in fertility between heifers and lactating cows. *Reproduction.2010 Jun*;*139 (6):1047-55;Epub 2010 Apr 12*.
10. Fouladi-Nashta AA, Gutierrez CG, Gong JG, Garnsworthy PC, Webb R. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. *Biol Reprod.2007 Jul;77(1):9-17*.
11. Abayasekara DR, Wathes DC. Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. *Prostaglandins Leukot Essent Fatty Acids.1999 Nov;61(5):275-87*.
12. Mattos R, Staples CR, Thatcher WW. Effects of dietary fatty acids on reproduction in ruminants. *Rev Reprod.2000 Jan;5(1):38-45*.
13. Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. *Reproduction.2001 Mar;121(3):447-54*.
14. Bilby TR, Block J, do Amaral BC, Sa Filho O, Silvestre FT, Hansen PJ et al. Effects of dietary unsaturated fatty acids on oocyte quality and follicular development in lactating dairy cows in summer. *J Dairy Sci.2006 Oct;89(10):3891-903*.

15. Mehdizadeh A, Rahimipour A, Farzadi L, Darabi M, Vatankhah A, Shaaker M et al. Correlation between the level of cholesteryl ester transfer protein in follicular fluid with fertilization rates in IVF/ ICSI cycles. *Iran J Reprod Med.2011;9(3):193-8*.
16. Jakab A, Kovacs T, Zavaczki Z, Borsos A, Bray-Ward P, Ward D et al. Efficacy of the swim-up method in eliminating sperm with diminished maturity and aneuploidy. *Hum Reprod.2003 Jul;18(7):1481-8*.
17. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.1959 Aug;37(8):911-7*.
18. Pezeshkian M, Noori M, Najjarpour-Jabbari H, Abolfathi A, Darabi M, Darabi M et al. Fatty acid composition of epicardial and subcutaneous human adipose tissue. *Metab Syndr Relat Disord.2009 Apr;7(2):125-31*.
19. Thyzel E, Siegling S, Tinneberg HR, Gotting C, Kleesiek K. Age dependent assessment of TFPI levels in follicular fluid of women undergoing IVF. *Clin Chim Acta.2005 Nov;361(1-2): 176-81*.
20. Sahmani M, Sakhinia E, Farzadi L, Najafipour R, Darabi M, Mehdizadeh A et al. Two common polymorphisms in the peroxisome proliferator-activated receptor gamma gene may improve fertilization in IVF. *Reprod Biomed Online.2011 Sep;23(3):355-60*.
21. Wathes DC, Abayasekara DR, Aitken RJ. Polyunsaturated fatty acids in male and female reproduction. *Biol Reprod.2007 Aug;77(2):190-201.*
22. Gadella BM, Evans JP. Membrane fusions during mammalian fertilization. *Adv Exp Med Biol.2011; 713:65-80.*
23. Aardema H, Vos PL, Lolicato F, Roelen BA, Knijn HM, Vaandrager AB et al. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. *Biol Reprod.2011 Jul;85(1):62-9.*
24. Nakanishi T, Koutoku T, Kawahara S, Murai A, Furuse M. Dietary conjugated linoleic acid reduces cerebral prostaglandin E(2) in mice. *Neurosci Lett.2003 May;341(2):135-8*.
25. Angelucci A, Garofalo S, Speca S, Bovadilla A, Gravina GL, Muzi P et al. Arachidonic acid modulates the crosstalk between prostate carcinoma and bone stromal cells. *Endocr Relat Cancer.2008 Mar;15(1):91-100.*
26. Wen X, Tozer AJ, Butler SA, Bell CM, Docherty SM, Iles RK. Follicular fluid levels of inhibin A, inhibin B, and activin A levels reflect changes in follicle size but are not independent markers of the oocyte's ability to fertilize. *Fertil Steril.2006 Jun;85(6):1723-29*.
27. Heinrichs SC. Dietary omega-3 fatty acid supplementation for optimizing neuronal structure and function. *Mol Nutr Food Res.2010 Apr;54(4):447-56*.