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Original Article

Does different BMI influence oocyte and embryo quality by inducing fatty acid in follicular fluid?

Parvaneh Mirabi [a], Mohammad Javad Chaichi [b], Sedighe Esmaeilzadeh [a, *], Seyed Gholam Ali Jorsaraei [a], Ali Bijani [c], Mahjobeh Ehsani [b]

[a] Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
[b] Faculty of Chemistry, University of Mazandaran, Babolsar, Iran
[c] Social Determinant of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

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ABSTRACT

Objective: We aimed to assess the effects of obesity-related follicular fluid (FF) fatty acids (FAs) on the number and quality of oocytes, good embryo quality rate, and pregnancy rate.
Materials and Methods: This prospective cohort study was conducted on 105 infertile women under the age of 38, who underwent intracytoplasmic sperm injection (ICSI) from March 2015 to October 2015. They were grouped into three body mass index (BMI) categories. The fatty acids composition of the FF was analyzed by GC–MS head space method. We studied the FAs correlation with BMI and ICSI outcomes.
Results: The distribution of fatty acids did not differ significantly in each BMI group, with the exception for stearic that was marginally significant ($p = 0.05$). The mean number of mature oocytes did not differ significantly between the BMI groups, the percent of Metaphase II (MII) oocytes was inversely associated with the BMI ($r_s = -0.21$, $p = 0.03$). Kruskal–Wallis test showed that the distribution of good quality embryos' percentages were different in at least two categories of studied BMI groups ($p = 0.009$, $p = 0.02$). The mean concentration of palmitic acid was higher in nonpregnant patients for all of the studied BMI classes ($p = 0.02$, $p = 0.03$, $p = 0.05$), however, stearic ($p < 0.001$) and linolenic acids ($p = 0.01$) were higher in nonpregnant normal weight patients.
Conclusion: Differences in BMI are not associated with the fatty acid composition of the FF. The FF fatty acid possibly affects the outcome of ICSI through the achievement of clinical pregnancy. Therefore, it is essential to provide patients with nutritional counseling before they use assisted reproductive techniques.

Introduction

Obesity and overweight are among the most serious public health challenges around the world [1].

In 2014, the World Health Organization (WHO) reported over 1.9 billion adults (age ≥ 18 years) to be overweight. About onethird of this population (600 million individuals) were obese [2]. A recent study estimated the prevalence rates of obesity and overweight in Iran as 36.5% and 33.3%, respectively. According to the WHO, however, > 50% of Iranian adults are overweight or obese [3]. In addition to its various negative health effects, obesity increases the prevalence of female infertility by two–three times. Moreover, obese women may take longer to get pregnant [4].

Some studies on *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have described the adverse effects of obesity on fertility, oocyte quality, embryo development, and implantation and pregnancy rates. They have also highlighted the higher need for gonadotropin doses among obese women [4–6] Meanwhile, other studies have not documented any associations between obesity and undesirable IVF/ICSI outcomes [7,8].

Numerous studies have reported a relationship between high body mass index (BMI) and poor oocyte retrieval, poor embryo quality, and decreased clinical pregnancy rates [9,10]. Petanovski et al [11] showed obesity to have a strong impact on

* Corresponding author. Babol-Torkmahale-Fatemeh Zahra Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, 4717647745, Iran.
E-mail address: sesmael@yahoo.com (S. Esmaeilzadeh).

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endocrine status, follicular synchrony, and IVF success in infertile women.

By contrast, other investigations have indicated similar implantation and clinical pregnancy rates among normal-weight, overweight, and obese infertile women who underwent ICSI [9,12,13].

Food intake and BMI can affect the blood serum and follicular fluid free FAs composition. Overweight and obesity change women's serum metabolic parameters, increase serum fatty acid levels, and affect the follicular fluid. As oocytes and embryos are both very vulnerable to changes in their microenvironment, such changes can lead to their defects [14,15].

In addition, higher free fatty acids and changes in adipokines caused by higher BMI can affect oocyte competence [4].

Recent data from animal model studies have pointed out to a relationship between saturated fatty acids (such as palmitic and stearic acid) and decreased fertilization, cleavage, and blastocyst rates [16–18].

Valckx et al [19] evaluated the fatty acid contents of the follicular fluid and reported phospholipid and cholesteryl-ester to have the highest concentrations (42% and 34% respectively). Nevertheless, BMI had the most considerable effect on nonesterified fatty acids. The researchers argued that the direct effects of such changes on oocyte metabolism decreased oocyte quality and disturbed embryo development.

Only a few studies have examined the relationship between BMI and follicular fluid fatty acids. Meanwhile, owing to their differences in designs, cut-off values of BMI, and sample sizes, these studies have yielded conflicting results. Therefore, the present study aimed to assess the effects of obesity-related follicular fluid fatty acids on the number and quality of oocytes, good embryo quality rate, and pregnancy rates.

Material and methods

This prospective cohort study was conducted from March 2015 to October 2015, on 105 infertile women under the age of 38, who underwent ICSI and embryo transfer at Fatemeh Zahra Infertility and Reproductive Health Research Center, Babol University of medical sciences, Iran. Exclusion criteria were > 38 years age, donor oocyte, gestational surrogacy, patients with an accompanying medical problem which may lead to abnormal BMI such as diabetes mellitus, hyperthyroidism, or hypothyroidism.

A written consent was obtained from each patient for the use of FF and the study design was approved by the Ethical Review Committee of Babol University of Medical Sciences.

All women underwent long protocol for controlled ovarian hyper stimulation (COH) after ovarian suppression by the subcutaneous injection of 0.1 mg gonadotropin releasing hormone analog (triptorelin, Diphereline, Ipsen Pharma Biotech, France) from the midluteal phase of the preceding cycle. A total of 75–150 IU recombinant human follicle stimulating hormone (rFSH, 75 IU GONAL-f, Merck Serono, Germany) were admitted daily until the average diameter of the leading follicle reached 18–20 mm. Then human chorionic gonadotropin (HCG) (Karma, Germany) 10,000 IU was administered. Oocyte retrieval was performed 36 hours after HCG administration and under transvaginal ultrasound guidance. Collected oocytes according to their morphology, were classified into three grades; Metaphase II (MII), Metaphase I (MI) and Germinal vesicles (GV) [20]. After removal of the oocyte, FF were immediately centrifuged (1000 *g*, minutes) and stored at −80 °C until analysis.

Retrieved oocytes were fertilized by ICSI and cultured until the blastocyst stage.

Embryos with, little or no fragmentation, and a zona pellucida that is not extremely thick or dark in appearance were classified as Grade A, embryos with equally-sized blastomeres, minor cyotplasmic fragmentation covering $\leq$ 10% of the embryo surface Grade B, and blastomeres of distinctly unequal size and moderate-to-significant cytoplasmic fragmentation covering >10% of the embryo surface Grade C [21,22].

To investigate the correlation of the follicular fluid fatty acids and BMI patients are classified to three groups based on the WHO cutoffs; normal weight ($18.5 \leq \text{BMI} < 25.0$ kg/m^2) , overweight ($25.0 \leq \text{BMI} < 30$ kg/m^2) and BMI ≥ 30.0 kg/m^2 as obese [19].

Extraction method

The methylation or esterification of fatty acids was carried out (according to the method reported by Patterson et al [24] with minor modification. Briefly, the biological samples were vortexed and the plasma proteins were precipitated using ice-cold acetone and placed at −20 °C for 15 minutes. After centrifugation of precipitated proteins, aliquots of hexane and water were added to the supernatant and the samples and shaking them gently for some minutes. The samples were then centrifuged and a 0.25 mL aliquot of phosphate buffer (0.2 M and pH = 9.0) and iodomethane in dichloromethane (10 %V/V) was added the upper phase (hexane) and samples were vortexed for 5 minutes to form Fatty acid methyl ester (FAME). The fatty acid composition of the women samples are analyzed by GC–MS head space method. The standard FAME samples were obtained from Supelco Inc. (Sigma-Aldrich, Missouri, USA). The derivatization reagent was iodomethane (Sigma-Aldrich, Missouri, USA). Hexane and dichloromethane were obtained from Merck (Darmstadt, Frankfurt, Germany).

Apparatus

Quantitative GC–MS analysis was determined by using a GC: Agilent7890 and MS: Agilent5975c (with an HB5column). A model 220 Corning pH meter was used to carry out the pH measurements. Centrifugation was carried out by Hettich (Model Universal2S).

Statistical analysis

Standard statistical procedures were carried out using the Statistical Package for Social Sciences (SPSS) version 21.0. Normality of quantitative variables was revealed by Kolmogorov–Smirnov test and Student *t* test was used in comparing quantitative variables. Descriptive analysis was conducted for each variable including frequencies, ranges, and percent of variable in simples. Multivariate analysis of variance (MANOVA) and Spearman correlation test were used to assess the quality of oocytes and embryos between different classes of BMI.

The distribution of fatty acids between BMI groups was compared by Kruskal–Wallis test. Student *t* test was used to compare the mean value of fatty acids among pregnant and nonpregnant patients. A $p < 0.05$ was considered statistically significant.

Results

A total of 105 infertile women mean aged 32 ± 5.6 years were included in the study. According to mentioned BMI categories 31.4% of the women had a normal weight, 43.8% were overweight, and 24.8% were obese.

Demographic and clinical characteristics of sampled patients are shown in Table 1. According to Table 1 no significant differences

were found among BMI groups in terms of age, educational status, type of infertility, menstrual pattern, and cause of infertility.

Infertility causes included female subfertility, male factor infertility, combined male/female infertility, and unexplained which was 19.3%, 39.4%, 11.5%, and 29.8%, respectively.

As shown in Table 2, the mean number of mature oocytes did not differ significantly between BMI groups, but the mean percent of MII oocytes were significantly different between the groups ($p = 0.01$). *Post hoc* Dunn Q test revealed that the mean percent of MII oocytes from the total number of retrieval oocytes were significantly different between the normal and obese ($p = 0.02$), overweight and obese ($p = 0.03$), and normal with overweight groups ($p < 0.001$). Moreover obesity was associated with fewer numbers of fertilized oocytes ($p = 0.009$).

The correlation between BMI and the percent of MII, MI, and GV oocytes was tested using the Spearman correlation test. The percent of M2 oocytes was inversely associated with the BMI ($r_s = -0.21$, $p = 0.03$), whereas no significance correlation was found for M1 and GV oocytes, respectively ($r_s = 0.126$, $p = 0.20$) ($r_s = 0.10$, $p = 0.31$).

According to multivariate analysis of variance (MANOVA) the quality of oocytes were statistically significant between different classes of BMI ($p = 0.04$). The mean percent of M2 oocytes was different between BMI groups ($p = 0.02$), however, there were no significant differences between M1 ($p = 0.61$) and GV ($p = 0.40$) oocytes.

Interestingly, Kruskal–Wallis test revealed that the distribution of good quality embryos' percentages (2B and 4A) were different in at least two categories of studied BMI groups ($p = 0.009$, $p = 0.02$). *Post hoc* comparisons showed a significant difference between normal weight and obese patients ($p = 0.01$, $p = 0.02$).

The distribution of fatty acids in the follicular fluid between BMI groups is presented in Table 3. It is apparent from this table that the distribution of fatty acids did not differ significantly in each BMI group, with the exception for stearic that was marginally significant ($p = 0.06$).

As Table 4 shows, the mean FF concentrations of fatty acids were compared among pregnant and nonpregnant groups in each BMI class. The mean concentration of palmitic was higher in nonpregnant patients for all of the studied BMI classes ($p = 0.02$, $p = 0.03$, $p = 0.05$), but stearic ($p < 0.001$) and linolenic acids ($p = 0.01$) were higher in nonpregnant normal weight patients. (Figure 1).

Discussion

The present study was designed to determine the effects of obesity-related follicular fluid fatty acids on oocyte and embryo quality. An initial objective of the project was to identify the mean concentration of fatty acids in the follicular fluid based on BMI categories. Except for stearic acid (in which marginally significant differences were observed between BMI categories), the distribution of fatty acids was not significantly different between various BMI groups. Likewise, Valckx et al [14] and Robker et al [25] demonstrated that the concentrations of follicular fatty acids had no significant relationships with BMI. By contrast, Valckx [19] found associations between obesity and variations in the fatty acid composition of the follicular fluid.

Food intake and BMI can affect the blood serum and follicular fluid FFA composition and decrease oocyte and embryo quality. They are thus regarded as potential risk factors for infertility. For instance, lipolysis can increase nonesterified fatty acid concentrations in the follicular fluid. This will, in turn, lead to reductions in oocyte competence, embryo quality, and embryo metabolism [19,26,27].

Warzych et al [28] reported high dietary n-3 fatty acid intake to exert significant effects on not only fatty acid concentrations in the follicular fluid, but also transcriptional abundance of the *EEF1A1* marker gene (as an indicator of oocyte competence).

It was hypothesized that high levels of saturated fatty acids could be cytotoxic and lead to cell death through necrosis and apoptosis. According to Cnop et al [29], although palmitic acid induced lipotoxicity, oleic acid could counteract such toxicity by enhancing triglyceride formation. Moreover, a strong relationship has been reported between mitochondrial fatty acid beta-oxidation and oocyte and thus embryo quality following nonesterified fatty acid exposure.

Furthermore, our data showed the mean concentration of stearic acid to be higher in the normal-weight group. Previous studies have reported the beneficial effects of high dietary stearic acid content on plasma low density lipoprotein (LDL)-cholesterol

Table 1
Demographic and clinical characteristics of sampled patients.

	18.5 ≤ BMI < 25.0 (n = 33)	25.0 ≤ BMI < 30.0 (n = 46)	BMI ≥ 30.0 (n = 26)	*p*
Age (y)	30.61 ± 5.6	31.33 ± 6.1	33.73 ± 5.1	0.11[a]
Education, n (%)				
Elementary education	7 (21.2)	10 (21.7)	9 (34.6)	
High school	13 (39.4)	25 (54.3)	11 (42.3)	0.35[b]
College education	13 (39.4)	11 (23.9)	6 (23.1)	
Infertility, n (%)				0.75
Primary	25 (75.8)	37 (80.4)	19 (73.1)	
Secondary	8 (24.2)	9 (19.6)	7 (26.9)	
Duration of infertility (y ± SD)	4.5 ± 4.0	4.81 ± 4.3	6.09 ± 4.4	0.34
Infertility cause, n (%)				
Unexplained	11 (33.3)	13 (28.3)	7 (26.9%)	
Tubal factor	3 (9.1)	3 (6.5)	0 (0%)	
Endometriosis	0 (0)	2 (4.3)	1 (3.8%)	0.66
Poly cystic ovary (PCO)	4 (12.1)	4 (8.7)	5 (19.2%)	
Male factor	14 (42.4)	19 (41.3)	9 (34.6%)	
Combined male and female factor	1 (3)	5 (10.9)	4 (15.4%)	
Menstruation, n (%)				
Regular	28 (84.4)	41 (89.1)	18 (69.2)	0.11
Irregular	3 (9.1)	5 (10.9)	7 (26.9)	
Oligomenorrhea	2 (6.1)	0 (0)	1 (3.8)	

ANOVA = analysis of variance; BMI = body mass index.
[a] One-way ANOVA test.
[b] Chi-square test.

Table 2
Patient's hormonal assessment and reproductive outcomes among BMI groups.

	18.5 ≤ BMI < 25.0 (mean ± SD)	25.0 ≤ BMI < 30.0 (mean ± SD)	BMI ≥ 30.0 (mean ± SD)	p^*
FSH (mIU/mL)	7.76 ± 3.6	7.14 ± 3.6	7.10 ± 3.2	0.62
LH (mIU/mL)	7.16 ± 4.7	5.90 ± 4.2	8.54 ± 5.6	0.04
Prolactin (ng/mL)	20.80 ± 13.2	26.82 ± 15	18.55 ± 9	0.02
Estradiol (pg/mL)	1480 ± 859	1640 ± 245	1500 ± 902	0.16
No. oocyte	8.24 ± 5.5	9.02 ± 6	8.68 ± 4.5	0.83
MII (%)[a,b,c]	84 ± 31	86 ± 24	65 ± 36	0.01
M1 (%)	7 ± 17	9 ± 21	12 ± 16	0.26
GV (%)	4 ± 16.5	5 ± 13	3 ± 7	0.69
No. embryo	5 ± 4.3	4.5 ± 4.06	3.3 ± 2.7	0.48
Good embryo [a] (4 A) (%)	0.23 ± 0.3	0.15 ± 23	0.05 ± 0.14	0.02
Good embryo [a] (2 B) (%)	0.29 ± 0.07	0.15 ± 0.25	0.29 ± 0.07	0.009
No. embryos/no. oocytes (%)	0.55 ± 30	50 ± 25	50 ± 26	0.66
No. good quality embryos/no. embryo (%)	0.91 ± 13	84 ± 26	76 ± 30	0.25
No. good embryo/no. oocytes (%)	51 ± 30	43 ± 24	34 ± 31	0.09
Gonadotropin dosage	2430 ± 1040	2650 ± 925	2573 ± 1029	0.46
Embryo transfer	1.6 ± 0.6	1.6 ± 0.6	1.5 ± 0.8	0.63
Implantation rate	21 ± 0.39	20 ± 0.37	27 ± 0.43	0.73
Pregnancy rate, n (%)	8 (24.2)	13 (28.3)	8 (30.8)	0.85

BMI = body mass index; FSH = follicle-stimulating hormone; GV = germinal vesicles; LH = luteinizing hormone; MI = metaphase I; MII = metaphase II; SD = standard deviation.
Values are expressed as mean ± SD or percent.
*Kruskal–Wallis test.
[a]Statistical significance ($p < 0.05$) between 18.5 ≤ BMI < 25.0 and BMI ≥ 30.0.
[b]Statistical significance ($p < 0.05$) between 25.0 ≤ BMI < 30.0 and 25.0 ≤ BMI < 30.0.
[c]Statistical significance ($p < 0.05$) between 25.0 ≤ BMI < 30.0 and BMI ≥ 30.0.

Table 3
Follicular fluid fatty acids distribution in BMI categories.

Fatty acid (ppm)	18.5 ≤ BMI < 25.0		25.0 ≤ BMI < 30.0		BMI ≥ 30.0		p^b
	Mean ± SD	Median (IQR)[a]	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Palmitic	29.14 ± 19.33	9 (5,32.5)	21 ± 17.09	9 (7,13)	13 ± 16	8 (6.27,10)	0.49
Palmtoleic	25 ± 14.10	22 (19.05,33)	38 ± 76	22.05 (9,29.25)	24 ± 21.03	22.25 (7,31.5)	0.81
Stearic	20 ± 4.2	5.40 (3.25,9.40)	16 ± 3.0	4.15 (2.20,9)	15 ± 4.46	3 (0.97,8.7)	**0.05**
Myristic	3.14 ± 3.53	1.2 (50,6.9)	2.43 ± 3.08	1.15 (0.77,1.65)	4 ± 3.60	1.75 (0.9,7.9)	0.33
Oleic	27 ± 46.29	8.20 (4.45,33)	23 ± 50	6 (3.2,30.25)	24 ± 48.35	6 (1.27,21)	0.25
Linolenic	6 ± 8.08	3 (0.47,9)	6.56 ± 4	9 (1.22,9)	5 ± 4.06	3.4 (0.75,8.5)	0.22
Arachidonic	12.12 ± 36.41	3 (1,9)	12 ± 39	1.3 (0.7,8.5)	18.45 ± 50.26	2 (1.10,9)	0.75
Linoleic	21 ± 58	6 (2.45,9.5)	30 ± 118	4 (2.4,5.9)	30 ± 68.15	5.15 (2,9.17)	0.76
Tricosanoic	14.38 ± 4.50	14 (13,16.05)	36.33 ± 112	14 (13,18)	14 ± 5	13.35 (11.25,14.12)	0.26

BMI = body mass index; SD = standard deviation.
[a] IQR = interquartile range.
[b] Kruskal–Wallis test.

levels. Meanwhile, palmitic, lauric, and myristic acids all have hypercholesterolemic effects [30,31].

Serum FFA concentrations are affected by the type and amount of dietary fat intake. As serum play a major role in the composition of follicular fluid, metabolic changes in serum can alter the biochemical compounds of follicular fluid. The growth and maturation of follicles are hence affected by blood metabolite concentrations [14,32,33].

In a prospective cohort study, Chavarro et al [34] revealed that dietary trans fatty acids might increase the risk of ovulatory infertility. Therefore, it is essential to provide patients with nutritional counseling before they use assisted reproductive techniques. One major limitation of our study was not evaluating the participants' nutritional status. Also we did not find the significant difference between BMI and pregnancy rate probably due to small sample size.

Furthermore, in our study, the mean number of oocytes did not differ significantly across the BMI categories. However, we observed poor quality of oocyte among obese women. Therefore, body weight may affect the developmental competence of oocytes. Moreover, the mean number of high-quality embryos was significantly different across the normal-weight and obese patients. Although some studies have confirmed poorer oocyte and embryo quality in obese women [13,19,35,36], others have failed to find any association between the two [4,37,38].

According to previous research, increased levels of plasma fatty acids and insulin resistance in insulin target tissues can result from obesity and elevated adipose tissue mass. The same factors were also reported to be associated with decreased sex hormone-binding globulin (SHBG) and increased triglycerides and an inflammatory marker in follicular fluid [4,39].

Insulin is also known to stimulate both steroidogenesis and luteinizing hormone (LH) receptor expression in the theca and granulosa cells of the ovary. Therefore, the overexpression of LH in obese women affects ovulation and oocyte maturation in this group [40].

Another important finding was that the mean value of saturated fatty acids (palmitic, stearic) and linoleic were significantly higher in nonpregnant patients and palmitic was higher in nonpregnant patients for all of the studied BMI classes.

These results are consistent with data obtained in most animal studies that reported saturated fatty acids, specially palmitic and stearic, inhibited fertilization and were cytotoxic at high concentrations [19].

Table 4
Assessment of fatty acids with the outcome of pregnancy in BMI categories.

	Fatty acid (ppm)	Pregnancy outcome				p^a
		Pregnant		Nonpregnant		
		Mean ± SD	Median(IQR)	Mean ± SD	Median(IQR)	
18.5 ≤ BMI < 25.0	**Palmitic**	7.35 ± 9.40	7(0.32,8)	36.11 ± 55	11(6,47.5)	**0.02**
	Palmitoleic	21.42 ± 18.44	22(6.23.5)	26 ± 13	22.20(19.25,36)	0.33
	Stearic	2.55 ± 3	2(0.35,3.32)	25.42 ± 4.7	7.6(4,11.5)	**<0.001**
	Myristic	1.03 ± 1.01	0.9(0.17,1.37)	4 ± 3.80	2(0.55,9)	0.08
	Oleic	20.51 ± 33.43	8(1.52,30.05)	29.01 ± 50.12	8.40(4.85,35)	0.52
	Linoleic	3.41 ± 3.37	2.60(0.52,6.45)	6.57 ± 9.07	6(0.4,9)	0.53
	Arachidonic	2.50 ± 3	2(0,3.5)	7 ± 9.07	6(0.4,9)	0.26
	Linolenic	3.13 ± 3	3.05(0.55,4)	26.60 ± 6.6	7.6(3.5,10.15)	**0.01**
	Tricosanoic	17 ± 6	14.20(13,22.5)	14 ± 4.09	14(13,16.5)	0.47
30.0	**Palmitic**	15.37 ± 32.21	8.6(4,9)	23 ± 40	9.1(7.5,17)	**0.03**
	Palmitoleic	62.15 ± 138	22(2.35,31)	28.04 ± 25	22.1(9.15,31.5)	0.38
	Stearic	22 ± 41.23	5.80(0.65,12.5)	13.23 ± 24.6	4.10(2.45,9)	0.85
	Myristic	3.46 ± 4	1.30(8,9)	2.02 ± 2.60	1.10(0.75,1.5)	0.32
	Oleic	15.15 ± 22.5	4.2(9,24)	26 ± 57.03	6(4,30.05)	0.36
	Linolenic	8.80 ± 0.44	9(8.5,9)	6 ± 4.3	9(1.20,9)	0.39
	Arachidonic	33 ± 81	5.05(0.75,9)	6.15 ± 11.39	1.30(0.65,8.80)	0.62
	Linoleic	19.20 ± 36.07	4.10(3.5,8.6)	34.10 ± 138.28	4(2.30,9.50)	0.49
	Tricosanoic	70 ± 195.7	14(13.20,20.5)	23.19 ± 50.27	14(13,17.5)	0.48
BMI ≥ 30.0	Palmitic	7 ± 7.6	4.40(1.32,9.35)	15.18 ± 18.12	8.50(7,10.02)	**0.05**
	Palmitoleic	30 ± 27	24(6.5,42.5)	21 ± 18.04	21.5(6.55,25.12)	0.21
	Stearic	5.13 ± 4	5.6(0.85,9)	19 ± 59.40	2.30(0.97,6.30)	0.72
	Myristic	4.31 ± 4	2.45(0.82,9)	3.46 ± 4	1.30(0.97,8.80)	0.53
	Oleic	21 ± 23.54	1(1.55,39.70)	25 ± 57	5(1.22,10.45)	0.39
	Linolenic	5.10 ± 4.33	6(0.72,8.85)	5 ± 4.12	3.40(6,8.70)	0.96
	Arachidonic	4.11 ± 4.09	2(0.72,9)	26.10 ± 61.48	2.20(1.10,9)	0.77
	Linoleic	34.16 ± 73.46	9(21.17,18)	27.56 ± 68	3.10(1.7,9)	0.19
	Tricosanoic	14 ± 3.51	14.20(10.12,17.5)	13.40 ± 4.60	13.05(11.25,14)	0.21

BMI = body mass index; IQR = interquartile range; SD = standard deviation.
[a] Student *t* test.

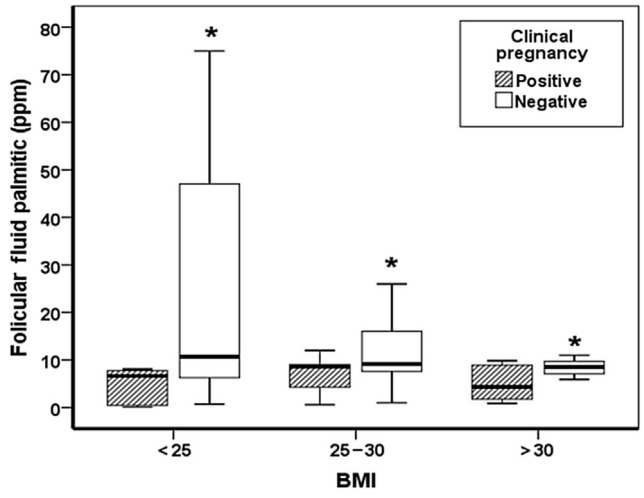

Figure 1. The mean value of palmitic in pregnant and nonpregnant patients based on BMI categories. BMI = body mass index. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Revelli et al [41] reported higher concentrations of linoleic acid in the follicular fluid of obese patients than in that of normal-weight patients.

A recent study found associations between BMI and the fatty acids content of follicular fluid. It thus concluded that obesity could affect oocyte maturation and developmental competence of embryos. However, contrary to our findings, no significant relationships were established between BMI and pregnancy and live birth rates [19].

Several published studies have demonstrated a positive correlation between high oleic acid content of follicular fluid and the fragmentation score of embryos. Meanwhile, stearic acid has been found to negatively affect the blastomere score among obese infertile women [33,42]. By contrast, McKeegan and Sturmey reported that the high concentration of palmitic acid elevated blastocyst formation and quality of embryos [16].

Another important finding of the present study was the high linolenic acid levels among nonpregnant patients. Linolenic acid is the most abundant polyunsaturated omega-3 fatty acid in bovine follicular fluid and the third in humans. Its concentration is critical for developmental processes and reproductive success. Haggarty et al [43] found significantly higher levels of linolenic and oleic acids in embryos that developed beyond the four-cell stage.

However, an animal model study showed an inhibitory effect of linolenic acid on oocyte competence, embryo cleavage, and blastocyst development [34].

Jungheim et al [44] detected reduced pregnancy rates in the presence of high levels of serum α-linolenic acid. However, controversial findings have been reported by other studies.

These observations may support the significance of the ratio and total concentration of each fatty acid during assisted reproductive technique.

Only a few studies have focused on the fatty acid contents of follicular fluid according to BMI classes. Due to the existing controversies in this regard, further research is required to determine the effects of diet and nutritional status on not only BMI-related fatty acid contents of follicular fluid, but also the number and quality of oocytes and embryos.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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