Theriogenology 108 (2018) 97–102

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effects of dietary omega-3 and -6 supplementations on phospholipid fatty acid composition in mice uterus during window of pre-implantation

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ARTICLE INFO

Article history:
Received 15 July 2017
Received in revised form
8 October 2017
Accepted 22 October 2017
Available online 29 November 2017

Keywords:
Embryo implantation
Pre-implantation window
Fatty acids
Omega-3
Omega-6
Arachidonic acid

ABSTRACT

Since fatty acid composition of uterus phospholipids is likely to influence embryo implantation, this study was conducted to investigate the effects of dietary omega-3 and -6 fatty acids on implantation rate as well as uterine phospholipid fatty acids composition during mice pre-implantation period. Sixty female mice were randomly distributed into:1) control (standard pellet), 2) omega-3 (standard pellet + 10% w/w of omega-3 fatty acids) and 3) omega-6 (standard pellet + 10% w/w of omega-6 fatty acids). Uterine phospholipid fatty acid composition during the pre-implantation window (days 1–5 of pregnancy) was analyzed using gas-chromatography. The implantation rate on the fifth day of pregnancy was also determined. Our results showed that on days 1, 2 and 3 of pregnancy, the levels of arachidonic acid (ARA) as well as total omega-6 fatty acids were significantly higher and the levels of linolenic acid and total omega-3 fatty acids were statistically lower in the omega-6 group compared to the omega-3 group ($p < 0.05$). On the fourth day of pregnancy, only the ARA, total omega-6 fatty acids, and polyunsaturated fatty acids levels were significantly different between the two dietary supplemented groups ($p < 0.05$). There were positive correlations between the levels of omega-6 fatty acids, especially ARA, with the implantation rate. The present study showed that diets rich in omega-3 and -6 fatty acids could differently modify uterine phospholipid fatty acid composition and uterine levels of phospholipid ARA, and that the total omega-6 fatty acids had a positive association with the implantation rate.

1. Introduction

For a successful implantation, the endometrium should undergo a series of processes to be appropriate for the embryo invasion. These processes take place during a specific period called the pre-implantation window [1]. Appropriate changes during this period can increase the receptivity of the endometrium and, consequently, enhance the chance of pregnancy [2,3]. However, one of the big challenges in infertility treatment is implantation failure [4].

Fatty acids, as the major compounds of endometrial cell membranes, have a vital role in embryo implantation [5]. These compounds are precursors of prostaglandins (PGs), which play a special role in endometrial receptivity by regulating inflammatory processes and the immune system [6]. Moreover, the composition of fatty acids in endometrial cell membranes may affect the biophysical properties of the uterus and, consequently, the interaction between the embryo and the uterus [7]. On the other hand, modified contents of gravid uterus and conceptus tissue fatty acids have been found following the modification of maternal

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https://doi.org/10.1016/j.theriogenology.2017.10.031
0093-691X/

supplementation [8]. The role of diet, as a life style modifiable factor in female fertility, has been investigated and also the effects of supplementation with poly-unsaturated fatty acids (PUFAs) on reproductive tissues have been discussed previously [9]. There is growing evidence about the determinant role of dietary amounts of PUFAs in female fertility, as positive influences of PUFAs on ovarian function [10], steroidogenesis [11], fertilization [12], embryo implantation [13] and pregnancy [14] have been widely documented. Abayasekara and Wathes [15] have mentioned that dietary PUFA affects fertility by changing the content of PUFAs in cell membranes and prostaglandin synthesis. On the other hand, any modification of endometrial fatty acids and changes in the omega-6:omega-3 fatty acid ratio has been observed as a consequence of the conceptus presence [16]. Experimental studies showed that flaxseed (rich in omega-3) supplemented cows compared to cows supplemented with soybean (rich in omega-6) or Megalac (rich in SFA) had higher conception and lower embryo changing the phospholipid fatty acid composition of the oocytes membrane. In a study on rats, it has been reported that different ratios of omega-6:omega-3 fatty acids could influence the morphological functional features of the endometrial and, hence, the success rate of embryo implantation [17].

To date, various mechanisms of action have been suggested for dietary PUFAs in embryo implantation and pregnancy such as the regulation PGs levels [18], modulation of the expression of uterine receptivity related genes [5], the providing of an energy source for decidualization [19], and the adjustment of the oxidative stress [20]. But so far, no study has been done to evaluate the effects of dietary PUFAs on the composition of uterus phospholipid fatty acids during the pre-implantation period. Since the fatty acid composition of uterus phospholipids is likely to influence embryo implantation through the above mentioned mechanisms as well as by changing the biophysical cell membrane properties, which, per se, affect the fusion of membranes between the blastocyst and uterus [7], this study was carried out to investigate the effects of dietary omega-3 and -6 fatty acids on the composition of uterus phospholipids in mice during the pre-implantation period. We also evaluated possible associations between fatty acids and the implantation rate.

2. Materials and methods

2.1. Animals and diets

Sixty two-month-old adult albino female NMRI mice and 20 male mice of the same strain were purchased from the RAZI institute, Iran. The average weight of the animals before the diet was 19.8 ± 4.6 g. All the animals were handled in accordance with the National Institutes of Health guide for the care and use of Laboratory animals and all procedures was approved by the Animal Ethical Committee of the Tabriz University of Medical Sciences. The females were randomly divided into three groups of 20 mice each: Normal (fed standard pellet manufactured by the RAZI institute, Iran), omega-3 (fed standard pellet + 10% w/w of omega-3 fatty acids), and omega-6 (fed standard pellet + 10% w/w of omega-6 fatty acids). An omega-3 supplement was provided as fish oil from the Danna Pharma Co. (Tabriz, Iran) and soybean oil (Italy) was used for omega-6 fatty acid supplementation. The fatty acid profiles of the standard chow pellet and supplemented fish and soybean oils were evaluated using gas-chromatography (data shown in Table 1).

The animals were housed under standard conditions of 25 ± 2 °C temperature, 60–70% humidity with 12 h light/dark cycle. Following the distribution of the animals into the groups mentioned above, they were fed the group-specific diet for three weeks. During this time, all the males were kept at the lab and fed standard pellet for adaptation. After three weeks of supplementation, three female mice of each group were placed overnight in a separate cage with a male mouse for natural mating. The next morning, the observation of a vaginal plug or spermatozoa in the vaginal smear was considered as the first day of the pregnancy and the pregnant mouse entered the study. The female mice were killed at 08:30–09:00 h of days 1–5 of pregnancy. For each day of pregnancy, in any group, four mice were sacrificed and the uteruses were collected and washed with phosphate buffered saline (PBS) and kept at −20 °C for fatty acid analysis. All mice and also their uterus was weighted on the day of sacrificing and uterine/body weight ratio × 1000 was calculated for each mouse. The implantation sites were determined on Day 5 of pregnancy using intravenous injection of 0.1 ml of 1% Chicago blue (Sigma Chemical Co., St. Louis, MO) in saline. For this purpose, the dye was injected via a tail vein after anesthesia based on instructions reviewed by Deb et al. [21]. About 5 min after dye injection, the mouse was sacrificed and blue bands were counted as implantation sites (Fig. 1). The

Table 1
Fatty acid profile of the standard chow pellet and also supplemented fish and soybean oils.

	Standard chow	Fish oil	Soybean oil
Meristic acid 14:0 (%)	1.13	0.32	1.80
Palmitic acid, 16:0 (%)	40.63	28.45	13.85
Palmitoleic acid, 16:1 (%)	1.43	5.87	0.22
Stearic acid, 18:0 (%)	6.83	6.70	3.05
Oleic acid, 18:1 (%)	28.86	21.78	25.79
Linoleic acid, 18:2 (%)	19.63	3.25	52.96
Linolenic acid, 18:3 (%)	1.49	2.08	2.24
Arachidonic acid, 20:4 (%)	–	3.55	<0.1
Eicosapentaenoic acid, 20:5 (%)	–	17.47	<0.1
Docosahexaenoic acid, 22:6 (%)	-	10.53	<0.1

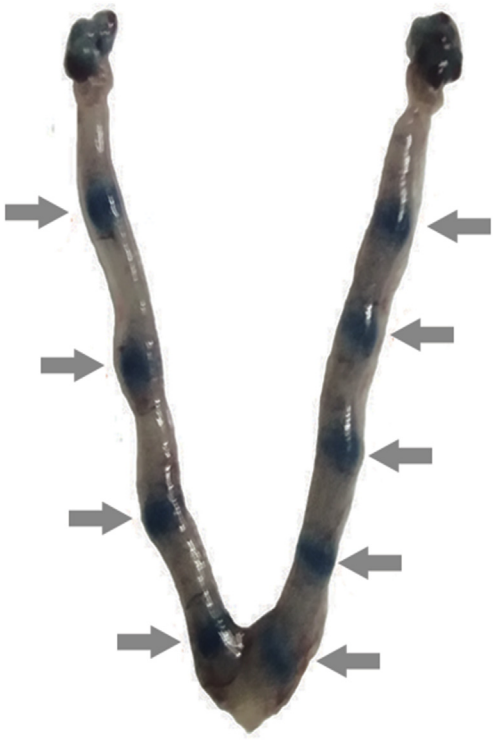

Fig. 1. Mice uterus at day 5 of pregnancy stained with Chicago blue dye. Implantation sites are shown with arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

number of implantation sites per uterus was considered as implantation rate.

2.2. Fatty acid analysis

The total lipids of the tissues were extracted according to the Bligh-Dyer method [22]. For this purpose, the tissue was first transferred into a glass tube containing 2:1 MeOH:chloroform solution and the tissue was smashed with a glass rod. The insoluble material was removed by centrifugation and the supernatant was mixed with chloroform and distilled water and shaken vigorously. After centrifugation, the chloroform phase was recovered and dried under a stream of nitrogen. Then the phospholipids were separated from the extracted lipids by thin-layer chromatography using a silica gel plate and a solvent system of hexane/diethyl-ether/glacial acetic acid (70:30:1). The phospholipid fraction was scraped in a glass tube containing hexane:methanol solution as well as tridecanoic acid (13:0, Sigma chemicals) as an internal standard. The fatty acids were esterified with methanol during catalysis with acetyl chloride (Lepage and Roy 1986), and fatty acid methyl ester derivatives were separated on a 60 × 0.25-mm Teknokroma TR CN100 column using a Buck Scientific model 610 gas chromatograph (SRI Instruments, Torrance, USA). In the gas chromatograph, helium and flame ionization were used as the carrier gas and detector respectively. The oven temperature was increased from 170 °C to 210 °C at the rate of 1 °C/min and then kept stable for 45 min. The known fatty acids from the Sigma chemicals were employed as standards of peak retention time identification. The levels of fatty acids were calculated as the percentage of each fatty acid of the total.

2.3. Statistical analysis

The skewness and Kurtosis tests were used to evaluate the data distribution. After confirming the normal distribution, parametric statistical methods were applied to analyze the data. One-Way ANOVA test following Tukey's post hoc test was employed to compare the quantitative parameters among various pregnancy days as well as different groups. The Pearson Correlation test was also applied to investigate possible associations between fatty acid levels and the implantation rate. Results with p-values <0.05 were considered statistically significant. SPSS V.16 software was used for the statistical analysis.

3. Results

There were no significant differences in body and wet uterine weights as well as the uterine/body weight ratio among the groups on the day of sacrificing ($p > 0.05$). Fatty acid compositions of uterine phospholipid of the control mice as well as omega-3 and -6 supplemented groups during the pre-implantation window are shown in Table 2. Our results showed that, on Day 1 of pregnancy, there was no difference in phospholipid fatty acid composition between supplemented groups and the control group ($p > 0.05$). However, on this day, the levels of arachidonic acid (ARA) as well as the total omega-6 fatty acids were significantly higher ($p = 0.021$ and 0.015, respectively) and levels of linolenic acid and total omega-3 fatty acids were statistically lower ($p = 0.016$ and 0.016, respectively) in omega-6 group compared to the omega-3 supplemented group ($p < 0.05$). In addition to the first day, on the second and third days of pregnancy, the same significant differences between the omega-3 and -6 supplemented groups were observed. Moreover, on Day 1, the levels of saturated fatty acids (SFA) and SFA to unsaturated fatty acids ratio (SFA/UFA) were higher in mice receiving dietary omega-3 compared to the omega-6 supplemented group ($p = 0.018$ and 0.027, respectively). On the third day of pregnancy, the linoleic acid levels were statistically lower in omega-3 supplemented mice compared to mice receiving omega-6 fatty acids ($p = 0.045$).

On Day 4 of pregnancy, the ARA and, consequently, the total omega-6 fatty acids and PUFA levels were significantly different between the two dietary supplemented groups ($p = 0.037$, 0.049 and 0.049, respectively), but no significant difference in omega-3 fatty acids levels was found between the groups ($p > 0.05$). On the fifth day of pregnancy, although levels of the omega-3 and omega-6 fatty acids were respectively higher and lower in omega-3 supplemented mice compared to the omega-6 group but the differences were not significant ($p > 0.05$).

The correlation of the implantation rate with phospholipid fatty acids levels in uterine tissues on the fifth day of pregnancy are listed in Table 3. In all the studied groups, there were positive correlations between the ARA levels and the implantation rate. The total levels of omega-6 fatty acids were also positively correlated with the implantation rate in control and omega-6 supplemented groups (r = 0970 and 0.964, respectively). We also observed a positive correlation between PUFAs levels and the implantation rate in the control group (r = 0.987). Moreover, in mice that received dietary omega-6, showed a significant positive correlation between linoleic acid levels and the implantation rate (r = 0.959).

4. Discussion

Effects of dietary fatty acids on pregnancy have been well documented. However, there is lack of information about the influence of dietary fatty acids on the composition of uterine phospholipid fatty acids and the implantation rate in early pregnancy. In the present study, we demonstrated that dietary omega-3 and -6 fatty acids could change the profile of uterus phospholipid fatty acids in different ways.

In the present study, we did not observe significant changes in the levels of palmitic, palmitoleic, stearic, and oleic fatty acids in uterine phospholipid following supplementation with omega-3 or -6 oils (Table 2). Similarly, it has been reported that dietary supplementation with fish had no effect on the endometrial composition of palmitic, palmitoleic, and oleic fatty acids in cows [23].

Although in our study as shown in Table 2, levels of linolenic acid were higher and levels of ARA and linoleic acid were lower in uterine tissue of omega-3 supplemented group compared to control group in almost all pre-implantation days, but the differences did not reach statistical significance possibly due to short-term of supplementation (three weeks). However, a comparison of omega-3 and -6 supplemented mice yield significant differences; as we observed a lower ARA and total omega-6 fatty acids and higher linolenic and total omega-3 fatty acid levels in mice receiving omega-3 supplemented diet. In accordance with our study, Howie et al. [24] have shown that three weeks of supplementation with omega-3-enriched diet increased omega-3 fatty acids incorporation into uterine lipids of rats compared to omega-6 supplemented diet. They also reported a lower amount of ARA in the uterine tissue of rats fed omega-3-enriched diet [24]. Decreased linoleic acid and increased ARA levels in endometrial tissue following fish meal supplementation have been also documented in cows [23]. Such an observed reduction in the ARA levels of uterine phospholipids in the omega-3 supplemented mice is possibly due to a decrease in ARA synthesis from linoleic acid, as some omega-3 fatty acids such as eicosapentaenoic and docosahexaenoic acids could inhibit the

Table 2
Fatty acid composition of uterine phospholipids of control, omega-3 and -6 supplemented mice during window of pre-implantation (days 1–5 of pregnancy).

	Day 1			Day 2			Day 3			Day 4			Day 5		
	Control	Omega-3 supplemented	Omega-6 supplemented	Control	Omega-3 supplemented	Omega-6 supplemented	Control	Omega-3 supplemented	Omega-6 supplemented	Control	Omega-3 supplemented	Omega-6 supplemented	Control	Omega-3 supplemented	Omega-6 supplemented
Palmitic acid, 16:0 (%)	25.24 ± 4.72	26.74 ± 4.04	22.85 ± 3.58	27.82 ± 4.44	28.74 ± 3.94	25.20 ± 2.80	29.48 ± 3.67	29.57 ± 4.03	26.67 ± 4.31	24.21 ± 3.30	26.17 ± 3.17	23.35 ± 4.52	21.83 ± 4.43	23.02 ± 3.43	18.91 ± 1.65
Palmitoleic acid, 16:1 (%)	3.52 ± 0.76	3.32 ± 0.75	3.35 ± 1.52	3.47 ± 1.03	3.88 ± 1.46	3.95 ± 1.24	3.72 ± 0.58	4.18 ± 1.56	4.02 ± 0.95	4.08 ± 1.25	3.90 ± 0.59	3.88 ± 1.58	3.90 ± 1.39	3.79 ± 1.27	4.44 ± 2.06
Stearic acid, 18:0 (%)	24.71 ± 4.58	24.33 ± 6.47	22.46 ± 3.07	27.00 ± 3.47	26.38 ± 2.94	24.96 ± 4.42	29.33 ± 3.91	29.06 ± 2.59	26.95 ± 5.63	24.79 ± 4.95	25.76 ± 4.40	23.19 ± 4.53	21.03 ± 3.52	22.21 ± 3.19	19.81 ± 2.55
Oleic acid, 18:1 (%)	21.40 ± 2.26	22.56 ± 4.91	21.12 ± 3.74	24.81 ± 3.46	24.65 ± 5.27	23.87 ± 4.63	24.84 ± 4.18	25.70 ± 6.27	25.31 ± 4.09	24.20 ± 3.31	26.52 ± 4.51	25.03 ± 3.78	23.02 ± 4.56	23.24 ± 3.60	24.92 ± 4.34
Linoleic acid, 18:2 (%)	11.45 ± 2.76	9.67 ± 1.70	13.32 ± 2.65	7.49 ± 0.76	6.26 ± 2.33	9.51 ± 2.10	5.29 ± 1.18	4.18 ± 0.89	6.73 ± 1.60[b]	10.28 ± 2.80	7.07 ± 2.27	10.56 ± 2.16	13.81 ± 2.78	11.54 ± 3.52	13.80 ± 2.84
Linolenic acid, 18:3 (%)	1.85 ± 0.46	3.08 ± 0.98	1.42 ± 0.41[b]	1.88 ± 0.17	3.66 ± 1.39[a]	1.55 ± 0.33[b]	2.46 ± 0.58	3.72 ± 0.77	1.97 ± 0.79[b]	2.42 ± 0.84	4.09 ± 1.39	2.20 ± 0.91	2.33 ± 0.40	3.06 ± 1.12	2.30 ± 0.78
Arachidonic acid, 20:4 (%)	12.00 ± 2.63	10.28 ± 1.02	15.46 ± 2.54[b]	7.54 ± 2.43	6.42 ± 1.65	10.94 ± 2.27[b]	4.95 ± 2.99	3.57 ± 0.96	8.58 ± 2.19[b]	9.82 ± 1.92	6.46 ± 2.05	11.77 ± 3.49[b]	14.20 ± 2.40	13.13 ± 3.39	15.79 ± 3.25
Omega-3 (%)	1.85 ± 0.46	3.08 ± 0.98	1.42 ± 0.41[b]	1.88 ± 0.17	3.66 ± 1.39[a]	1.55 ± 0.33[b]	2.46 ± 0.58	3.72 ± 0.77	1.97 ± 0.79[b]	2.42 ± 0.84	4.09 ± 1.39	2.20 ± 0.91	2.33 ±0.40	3.06 ± 1.12	2.30 ± 0.78
Omega-6 (%)	23.46 ± 2.86	19.95 ± 1.47	28.79 ± 5.11[b]	15.04 ± 1.71	12.68 ± 3.97	20.46 ± 4.13[b]	10.24 ± 3.28	7.75 ± 1.74	15.31 ± 3.74[b]	20.32 ± 3.01	13.54 ± 4.29	22.32 ± 5.62[b]	28.01 ± 5.18	24.67 ± 6.90	29.60 ± 6.07
Saturated fatty acids (%)	49.96 ± 2.91	51.08 ± 2.64	45.31 ± 1.04[a,b]	54.82 ± 1.82	55.13 ± 6.86	50.17 ± 1.95	58.82 ± 4.66	58.64 ± 6.60	53.63 ± 1.56	49.00 ± 2.81	51.94 ± 7.29	46.55 ± 1.92	42.87 ± 1.37	45.23 ± 6.59	38.73 ± 3.54
Mono-unsaturated fatty acids (%)	24.93 ± 1.93	25.89 ± 4.80	24.48 ± 5.12	28.28 ± 3.11	28.53 ± 6.69	27.81 ± 5.77	28.56 ± 4.15	29.88 ± 7.38	29.33 ± 5.00	28.28 ± 3.73	30.42 ± 4.78	28.91 ± 5.31	26.91 ± 4.48	27.03 ± 3.25	29.37 ± 0.5.73
Poly-unsaturated fatty acids (%)	25.31 ± 2.59	23.03 ± 2.28	30.20 ± 5.48	16.92 ± 1.72	16.34 ± 2.64	22.01 ± 3.98	12.70 ± 2.71	11.48 ± 1.17	17.28 ± 4.51	22.74 ± 2.25	17.63 ± 2.91	24.53 ± 4.78[b]	30.28 ± 5.20	27.73 ± 5.83	31.90 ± 5.31
Ratio of saturated to unsaturated fatty acids (%)	1.00 ± 0.12	1.04 ± 0.11	0.83 ± 0.03[b]	1.21 ± 0.08	1.27 ± 0.38	1.01 ± 0.08	1.45 ± 0.30	1.47 ± 0.44	1.15 ± 0.06	0.96 ± 0.10	1.12 ± 0.34	0.87 ± 0.06	0.75 ± 0.04	0.84 ± 0.23	0.75 ± 0.04

a,b Different superscripts within columns indicate differences ($p < 0.05$) between groups.

conversion of linoleic acid into ARA by occupying the responsible desaturase and elongase enzymes [25]. Another reason for the low ARA levels in uterine phospholipids of mice getting omega-3 could be due to a reduction in ARA uptake by the cells due to competitive displacement of omega-3 fatty acids such as eicosapentaenoic acids instead of ARA. In keeping with this explanation, an in-vitro study on human endometrium tissue has demonstrated that adding eicosapentaenoic acid to the culture medium could decrease incorporation of ^{14}C-ARA into the phospholipids [26]. On the other hand, it had been documented that receiving omega-3 enriched diet could significantly increase eicosapentaenoic acid levels in plasma and ovaries [27] as well as endometrium [28]. Decreased ARA and linoleic acids levels in the endometrial tissue of bovine supplemented with dietary n-3 PUFAs had also been reported earlier [18], which is in accordance with our findings, especially on the third day of pregnancy.

It has been suggested that modification of endometrial fatty acids could be considered as a possible way to assist early pregnancy [29,30]. Our finding about significantly higher implantation rate in mice that received omega-6 compared to the omega-3 group could somewhat confirm the hypothesis. However, in the present study, although on the implantation day the levels of ARA and linolenic acids were respectively higher and lower in the omega-6 group than mice that received omega-3-rich diet, the differences were not statistically significant. Such results could be due to the presence of embryo, as it has been reported that the conceptus could affect the endometrial fatty acids profile in favor of omega-6 fatty acids, especially ARA [16,31]. So it could be postulated that possibly the presence of an embryo in the uterus increased the phospholipids omega-6 fatty acids levels, especially that of the ARA, and thus compensated the effect of diet in the omega-3 group. It should also be noticed that, in the present study, we had just three weeks of supplementation with only 10% w/w of PUFAs, so further studies with long-term and high ratio of PUFAs supplementation are required to reach an exact conclusion.

We found a higher rate of implantation in mice fed with omega-6 fatty acids compared to those given omega-3. There were also positive correlations between phospholipid ARA levels as well as total omega-6 fatty acid levels and the implantation rate (see Table 3). There are at least two possible explanations for a higher implantation rate among mice getting omega-6 compared to the omega-3 supplemented group and also the association between phospholipid omega-6 fatty acids, especially ARA, with the implantation rate: 1) *Through affecting PGs*: ARA could lead to the production of a series 2 PGs, more functionally active than series 3 PGs produced by omega-3 fatty acids [29]. Increase and decrease in the levels of prostaglandin F2 (PGF2) and prostaglandin E2 (PGE2) in human decidual cells following omega-6 and -3 supplementation, respectively, has been reported [32]. It has been demonstrated that fish oil supplementation could decrease gene expression of the PGF synthase (PGFS) and PGE synthase (PGES) in bovine endometrium [18,33]. We also observed that omega-6 PUFA supplementation could increase the expression of PGs synthesis enzymes as well as PG receptors in mice uterus [34]. On the other hand, crucial roles of series 2 PGs in embryo implantation and decidualization have been well documented, as it has been found that PGE2 and PGI2 induce permeability of vessels and decidualization at implantation sites [35]. An in-vitro study has also confirmed the importance of PGE2, PGF2 and their receptors (EP2 and FP) in embryonic adhesion [36]. In mice, the prevention of PG synthesis could cause implantation failure [37]. Moreover, a positive association between PGE2 levels in the uterine fluid with embryo growth and maintenance has been shown [38]. 2) *Through altering the steroid hormone levels and receptors*: Increased serum progesterone levels in the late luteal

Table 3
Correlation of implantation rate with fatty acid composition in uterine tissue on embryo implantation day (day 5 of pregnancy) in control, omega-3 and -6 supplemented groups.

	Implantation rate					
	Control group		Omega-3 group		Omega-6 group	
	r	*p*	r	*P*	r	*p*
Palmitic acid, 16:0 (%)	−0.149	0.851	−0.617	0.383	0.630	0.370
Palmitoleic acid, 16:1 (%)	0.140	0.860	−0.404	0.596	−0.523	0.477
Stearic acid, 18:0 (%)	−0.069	0.931	−0.569	0.431	−0.451	0.549
Oleic acid, 18:1 (%)	−0.750	0.440	0.197	0.803	−0.926	0.074
Linoleic acid, 18:2 (%)	0.890	0.070	0.666	0.334	0.959	**0.041**
Linolenic acid, 18:3 (%)	−0.099	0.901	−0.852	0.148	−0.939	0.061
Arachidonic acid, 20:4 (%)	0.989	**0.011**	0.953	**0.047**	0.974	**0.026**
Omega-3 (%)	−0.099	0.901	−0.852	0.148	−0.939	0.061
Omega-6 (%)	0.970	**0.030**	0.937	0.063	0.964	**0.036**
Saturated fatty acids (%)	−0.660	0.340	−0.597	0.403	−0.031	0.969
Mono-unsaturated fatty acids (%)	−0.948	0.052	0.060	0.940	−0.890	0.110
Poly-unsaturated fatty acids (%)	0.987	**0.013**	0.641	0.359	0.932	0.068
Ratio of saturated to unsaturated fatty acids (%)	−0.632	0.368	−0.531	0.469	−0.035	0.965

phase of cows that received omega-6 diet compared to those getting omega-3 has been observed [39]. On the other hand, progesterone, as the most important hormone in the maintenance of pregnancy, can also prepare the endometrium for embryo implantation possibly by changing the endometrial morphology [17]. Moreover, it has been shown that the luteinizing hormone (LH) increases ARA release from cell membrane of steroidogenic cells and, consequently, the ARA could induce steroid hormone production possibly by altering the expression of the steroidogenic acute regulatory (StAR) gene [40,41]. However, there are studies that report no differential influences of omega-3 and -6 fatty acids on steroid hormone production [42] or positive effect of omega-3 fatty acids on progesterone levels [43]. Therefore, further studies are needed in order to understand the exact effects of PUFAs on steroid hormones and the underlying mechanisms.

In accordance with our findings, it has been demonstrated that ARA and its metabolites are crucial for the initiation of implantation via the epidermal growth factor (EGF) [44]. It has also been documented that the treatment of endometrial cells with omega-6/omega-3 ratio of 4 has a positive influence on genes that are involved in uterine receptivity and embryo implantation. Although in this study a ratio of 25 did not show such effect, implying the importance of balance between omega-3 and -6 fatty acids [5]. Moreover, the adverse effects of high omega-3 fatty acids on ovulation frequency [45], zygote morphology [27], embryo development [27] and endometrial cells survivability [46] have been reported. However, in contrast with our results, it has been shown that cows fed omega-3 PUFAs had fewer pregnancy loss and embryo mortality and a higher conception rate than cows that received omega-6 fatty acids [47–49]. Such a contrast could be due to a difference in the studied species. Moreover, since implantation is considered as a pro-inflammatory process [50] and omega-6 fatty acids, unlike omega-3 fatty acids, have inflammatory effects, it seems, at least in the pre-implantation period, high levels of omega-6 fatty acids would favor embryo implantation.

In conclusion, the present study showed that diets rich in omega-3 and -6 fatty acids could differently modify the phospholipid fatty acid composition of mice uterus. Moreover, we found that omega-6 supplementation had positive effect on the implantation rate in mice. We also observed a positive association of the uterine levels of phospholipid ARA and total omega-6 fatty acids with the implantation rate.

Conflicts of interest

None.

Acknowledgments

The study was financially supported by the Women's Reproductive Health Research Center, Tabriz University of Medical Sciences (grant number of 949/14.07.15) and the paper was extracted from PhD dissertation of Amir Fattahi.

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