REVIEW

The association between fatty acid index and in vitro fertilization outcomes

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Abstract

Purpose Fatty acids have been shown to play an important role in oocyte competence and early implantation of the embryo. Our hypothesis-generating study sought to determine if individual fatty acids expressed as a percentage of total erythrocyte fatty acids are associated with embryo quality and other in vitro fertilization (IVF) outcomes.

Methods This was a prospective cohort study at an academic fertility center. Sixty women undergoing their first IVF cycle were recruited. Serum measurements of 22 fatty acids were obtained. We calculated each fatty acid as a percentage of total fatty acids, defined as the index for that individual fatty acid. **Results** Omega-3 index had no correlation with IVF outcomes. A negative correlation was found between the trans fatty acid index, elaidic acid (EA), and IVF outcomes, including fertilization rate ($r = -0.261$, $p = 0.04$), blastocyst conversion rate ($r = -0.41$, $p = 0.001$), and number of usable blastocysts and embryos ($r = -0.411$, $p = 0.001$). There was no correlation between EA index and number of oocytes retrieved, embryo grade, or clinical pregnancy. No consistent correlations were observed with the additional fatty acids analyzed.

Conclusions No correlation was observed between omega-3 index and IVF outcomes. Elevated erythrocyte EA index, the major trans fatty acid commonly consumed in hydrogenated oils, margarine, and fried foods, was negatively correlated with number of usable blastocysts and embryos, blastocyst conversion, and fertilization rate. Our findings suggest preliminary evidence that trans fat may be negatively associated with IVF outcomes.

Keywords Omega-3 index · Trans fat · Fatty acid index · Blastocyst · In vitro fertilization

Introduction

Fatty acids are known to be important substrates of early reproductive events such as oocyte maturation and embryo implantation. Polyunsaturated fatty acids (PUFAs) are essential components of membrane lipids, which increase rapidly with each cell division [1, 2]. Fatty acids act as the building blocks that help regulate endocytosis/exocytosis and ion-channel modulation and directly stimulate enzymes that promote cell growth and differentiation [3]. They act as necessary precursors for prostaglandin synthesis and potentially as ligands for peroxisome-proliferator-activated receptors (PPARs) which are responsible for fatty acid storage throughout the body and at the level of the ovary [4, 5].

Omega-3 fatty acids have long been established as essential elements of the human diet necessary for normal growth and development. Their supplemental role in improvement and management of a number of chronic disease states including cardiovascular health, diabetes, renal disease, and autoimmune phenomena is well established [6, 7]. Omega-3 fatty acids are generally considered to be hypolipidemic, antithrombotic, and anti-inflammatory and are frequently taken as

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supplements in the form of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) secondary to modern dietary deficiencies [8, 9].

The impact of omega-3 fatty acid supplementation on fertility and IVF outcomes is less well established, and evidence is conflicting [4, 10–13]. In a prospective cohort of women undergoing IVF with intracytoplasmic sperm injection (ICSI), increased dietary intake of α -linolenic acid (ALA) and DHA in the 4 weeks preceding their stimulation cycle was associated with improved embryo morphology [10]. This study was limited by lack of serum or tissue level measurements, and intake was quantified solely by dietary recall. Another prospective randomized control trial comparing a multi-nutrient supplement containing omega-3 versus folic acid alone suggested improved embryo quality with addition of the multi-nutrient supplement [11]. These results could have been confounded by potential beneficial effects of additional nutrients contained in the supplement. In contrast, elevated free fatty acids (FFA) have also been shown to correlate with poor cumulus oocyte morphology and oocyte competence. Increasing amounts of ALA were specifically shown to be associated with a decreased implantation rates and decreased occurrence of a clinical pregnancy thought to be due in part by aberrant PPAR function involved in both embryonic and placental development [4, 12, 13].

Trans fatty acids, a type of unsaturated fat with at least one double bond in the trans configuration, are uncommon in nature but commonly produced industrially. Trans fats are most often found in partially hydrogenated vegetable oils, margarines, and fried food and are added to packaged prepared foods to increase shelf life [9]. They occur naturally in small amounts in dairy products and meats and are present in low levels in other edible oils. Trans fat has been shown to have a dose-dependent increase in the risk of cardiovascular disease and type 2 diabetes as well as contributing to a pro-inflammatory state in the human body [9, 14–16]. Studies regarding the influence of trans fats on an individual's reproductive potential have shown that increasing levels are associated with higher rates of fetal loss and implantation failure as well as ovulatory dysfunction through alteration of PPAR- γ receptors in women [17–19]. An inverse relationship between trans fat intake and sperm concentration and fertilization rate in men has also been demonstrated [20, 21]. The impact of trans fat directly on IVF outcomes including number of oocytes retrieved and embryo quality is less well described.

Erythrocyte fatty acid levels have been established as surrogate measure for follicular fluid levels of fatty acids in prior studies [4, 13]. The aim of this hypothesis-generating study was to determine if the erythrocyte content of 22 fatty acids expressed as a percentage of total fatty acids correlates with oocyte yield, embryo development, and clinical pregnancy rates in women undergoing IVF.

Materials and methods

Carolinas Medical Center Institutional Review Board approved all study protocols. This was a prospective cohort study at a single academic medical center that enrolled infertile women undergoing IVF from April 2012 to January 2013. Inclusion criteria were women age 40 or younger, first IVF cycle, normal uterine cavity, antimüllerian hormone level greater than 1.0 ng/mL, and patient weight of 50 kg or greater.

All subjects underwent an oral contraceptive leuprolide stimulation cycle for IVF. Serial monitoring of ovarian response was assessed by transvaginal ultrasound and serum estradiol (E2) assays. Treatment began with oral contraceptive pills and leuprolide; leuprolide was continued until the day of human chorionic gonadotropin (hCG) administration. A standard starting dose of gonadotropins (225 IU/day follicle-stimulating hormone) and concurrent leuprolide were given for 8–10 days, with follicular scans and E2 levels on days 4, 6, and 8 prior to hCG administration. For uniformity, blood from stimulation day 8 was drawn and frozen for fatty acid index assay. When a patient had two or more follicles ≥ 18 mm, she was given hCG. Oocyte retrieval took place 36 h later (day 0) and either conventional insemination or ICSI was performed. Fertilization was assessed on day 1, and embryos were then assessed daily. Embryo transfer was performed on day 3 or 5. Increasing beta-hCG levels 2 weeks after embryo transfer were considered evidence of pregnancy. A viability ultrasound was performed at 6–7 weeks gestational age to confirm a viable intrauterine pregnancy, defined as clinical pregnancy.

Embryo and blastocyst grading was performed by a single embryologist blinded to the fatty acid index results. Grading on day 3 was based on cell division and fragmentation. Grade 1 cleaving embryos had blastomeres of equal size with no fragmentation. Grade 2 embryos had blastomeres of equal size and minor cytoplasmic fragmentation of less than 10% of the pre-embryo. Grade 3 embryos had blastomeres of unequal size and variable fragmentation. Grade 4 embryos had blastomeres of equal or unequal size and moderate-to-significant cytoplasmic fragmentation of $> 10\%$ [22]. Number of usable blasts is the number of blastocysts transferred or frozen in that cycle. Embryos must be grade 3 at minimum to transfer or freeze in our institution.

Fasting blood draw for erythrocyte fatty acid composition was performed on cycle day 8 per protocol concurrent with an estradiol level. Erythrocytes were isolated from blood drawn and frozen at -80°C immediately after collection. Erythrocyte fatty acid composition was analyzed by gas chromatography with flame ionization detection by a standardized, validated method, which has been described previously [23, 24]. Unwashed, packed RBCs (25 μL) were transferred to a screw-cap glass vial. Methanol containing 14% boron trifluoride (Sigma-Aldrich, St. Louis, MO) and hexane

(EMD Chemicals, USA) was added sequentially, 250 μ L of each. The vial was briefly vortexed and then placed in an aluminum bead hot bath at 100 °C for 10 min. After cooling, 250 μ L of HPLC grade water was added; the tubes recapped, vortexed, and centrifuged for 3 min at 3000 RPM to separate layers. A 50- μ L aliquot of the hexane (upper) layer was transferred to a GC vial. GC was carried out using a GC2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP2560, 100-m fused silica capillary column (0.25-mm internal diameter, 0.2- μ m film thickness; Supelco, Bellefonte, PA). Run conditions were carrier gas, hydrogen; linear velocity, 22.0 cm/s; injector temperature, 230 °C; oven program, initial temperature of 140 °C hold for 5 min, ramp temperature at a rate of 4 °C/min to 240 °C and hold for 15 min (total run time 45 min); and detector temperature, 240 °C. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of erythrocytes (GLC 727, NuCheck Prep, Elysian, MN) that were also used to determine individual fatty acid response factors. The following 22 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 24:0); *cis* monounsaturated (16:1, 18:1, 20:1, 24:1); *trans* (16:1, 18:1, 18:2); *cis* n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); and *cis* n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). The fatty acid index (FAI), the assay that was used in this study, expresses each fatty acid as a percentage of total erythrocyte fatty acids as determined by standardized methodology and has been shown to be a means to measure numerous clinical outcomes [23–26]. The omega-3 index is expressed as the sum of EPA and DHA. Bonferroni correction was employed to correct for multiple comparisons with the use of 27 test variables.

Statistics

The relationship between each of the individual fatty acids and omega-3 index with number of oocytes retrieved, fertilization rate, percentage of grade 1 or 2 embryos on day 3, blastocyst conversion rate, and clinical pregnancy was analyzed. Blastocyst conversion rate describes the number of blasts from 2 pronuclear (2pn) embryos. Fertilization rate is the number of 2pn embryos per number of mature oocytes.

Descriptive statistics including means and standard deviations were calculated. Student's *t* test was used for data measured on an interval scale including age and BMI when comparing women who became pregnant versus those who did not. Chi-square or Fisher's exact test was used for categorical data. If the data were ordinal or not normally distributed, Wilcoxon rank sum test was employed. When the outcome was measured on the interval scale, the groups were compared using analysis of variance or the Kruskal-Wallis test if the data were not normally distributed including diagnosis subgroups. For the dependent variables, blastocyst conversion rate and

embryo grade, a two-way analysis of variance was performed. Pearson's correlation coefficients were used to test for linear relationships among the variables. SAS®, version 9.2, was used for all analyses. Eighty percent power was used to detect a difference in embryo development. To correct for multiple comparisons, Bonferroni correction was calculated to establish a more stringent threshold of a two-tailed *p* value less than 0.0019 (0.05/27 test variables) to be considered statistically significant.

Results

Of the 60 patients enrolled, 70% were Caucasian, 15% African American, 1.6% Asian Indian, and 13.3% other race/ethnicity. Mean age was 33.1 years and mean BMI was 26.8 kg/m². Diagnoses of tubal factor, male factor, ovulation disorder, unexplained infertility, or other (including recurrent pregnancy loss and uterine factor) were included [Table 1].

The omega-3 index among patients ranged from 2 to 10% (mean 6%, SD 2%). When categorized into tertiles of omega-3 index of 0–4.5% (*n* = 18), 4.5–6.5% (*n* = 24), and > 6.5% (*n* = 18) to assess for dose-dependent response, no correlation was seen with BMI (*p* = 0.08), age (*p* = 0.3), number of oocytes retrieved (*p* = 0.23), or mean number of oocytes fertilized (*p* = 0.25). Additionally, no correlation in the mean percentage of grade 1 and 2 embryos (*p* = 0.854), blastocyst conversion rate (*p* = 0.9), and fertilization rate (*p* = 0.984) was observed. For those embryos transferred, there was no difference in implantation rates between omega-3 index groups [0–4.5% (*n* = 8), 4.5–6.5% (*n* = 7), > 6.5% (*n* = 8)]; *p* = 0.699 [Table 2]. Comparing those patients with a clinical pregnancy (*n* = 31) to those with no clinical pregnancy (*n* = 29), there was no difference in omega-3 index (*p* = 0.40). When comparing omega-3 index and etiology of infertility, no variance was found between the subgroups.

Elaidic acid index ranged from 0.37–1.70%. There was no correlation between age (*p* = 0.71) or BMI (*p* = 0.24) and elaidic acid index. Elaidic acid index was not associated with infertility subgroup (*p* = 0.85). We found a negative

Table 1 Demographic and clinical characteristics of all study participants

Characteristic	<i>n</i> = 60
Age (years) ^a	33.1 (3.57)
BMI (kg/m ²) ^a	26.8 (5.94)
Diagnosis <i>n</i> (%)	
Tubal factor	10 (16)
Male factor	24 (40)
Ovulatory	10 (16)
Unexplained	11 (18)
Other	5 (6)

^a Values are mean $\pm$ (SD)

Table 2 Omega-3 index and IVF outcomes

Omega-3 index	0–0.045 <i>n</i> = 18	0.045–0.065 <i>n</i> = 24	> 0.065 <i>n</i> = 18	<i>p</i> value ^a
Number of oocytes retrieved	17.6 (5.4)	15.1 (7.3)	17.5 (7.4)	0.23
% Grade 1 + 2 embryos	58.76 (26.9)	54.46 (21.1)	56.09 (26.1)	0.85
Blastocyst conversion rate	0.37 (0.26)	0.33 (0.25)	0.36 (0.28)	0.9
Fertilization rate	0.63 (0.17)	0.62 (0.19)	0.63 (0.18)	0.98

Values are mean ± (SD)

^a *p* values are unadjusted

correlation between the erythrocyte elaidic acid index and IVF outcomes including fertilization rate ($p = 0.04$, $r = -0.261$), blastocyst conversion rate ($p = 0.001$, $r = -0.411$), and decreased number of usable blastocysts and embryos ($p = 0.001$, $r = -0.411$) [Fig. 1]. Correlation between blastocyst conversion rate and number of usable blastocyst and embryos remained significant following Bonferroni correction. No trend was observed when comparing the EA index to number of oocytes retrieved ($p = 0.78$), embryo grade ($p = 0.61$), or clinical pregnancy ($p = 0.62$) [Table 3].

Of the additional fatty acids analyzed, no consistent significant correlations were observed [Supplemental Table 1].

Comment

In this study of women undergoing IVF, fasting serum fatty acid index levels drawn on day 8 of gonadotropin cycle suggests preliminary evidence that elaidic acid index may be negatively associated with fertilization rate, blastocyst conversion rate, and number of usable blastocysts and embryos. No correlation was observed between omega-3 index or the additional fatty acids analyzed and other outcome measures including number of oocytes retrieved, embryo grade, or clinical

pregnancy rate. While these findings are thought provoking, our results require confirmation in a more comprehensive study.

Prior studies have demonstrated that erythrocyte fatty acid content strongly correlates with ovarian follicular fluid content and that intake has a causative effect on the follicular micro-environment [4, 13]. It has been suggested that higher amounts of omega-3 fatty acids would improve membrane fluidity and result in less embryo fragmentation and more symmetric division of blastomeres, factors that are associated with improved implantation rates and higher live birth rates, as well as an improved blastocyst development [2, 10, 22]. We found no trend between embryo morphology or pregnancy rate and erythrocyte omega-3 index. EPA and DHA are long-chain fatty acids (LCFA) most commonly found in commercial omega-3 nutritional supplements. Some studies suggest that over supplementation or high levels of omega-3 directly from dietary intake could in fact be harmful as these lipids are extremely sensitive to oxidation and may result in detrimental oxidative stress of gametes and embryos [27]. Specific genetic variants of enzymes involved in lipid metabolism have been identified that alter metabolism of omega-3 among different populations, suggesting that supplementation and the resultant supra-physiologic levels cannot be universally applied and could be particularly concerning in couples

Fig. 1 Elaidic acid index and fertilization and blastocyst conversion rate

Table 3 Elaidic acid index and IVF outcomes

Elaidic acid index (0.37–1.70%) (<i>n</i> = 60)	<i>p</i> value ^a	Pearson's (<i>r</i>)
Number of oocytes retrieved	0.785	− 0.036
% Grade 1 + 2 embryos	0.606	− 0.068
Fertilization rate	0.04	− 0.261
Number of usable blastocysts and embryos	0.001 ^b	− 0.411
Blastocyst conversion rate	0.001 ^b	− 0.41

^a *p* values are unadjusted

^b *p* < 0.0019, which we considered the level of significance after using Bonferroni correction

seeking pregnancy [28]. The preliminary data we present here may not suggest a supplemental benefit from omega-3 fatty acids on embryo development.

Trans fats have been shown to increase rates of fetal loss, endometriosis, and ovulatory dysfunction in females and decreased fertilization rates and sperm concentration in males [17, 19–21, 29]. Work done previously with the Nurses' Health Study II showed that increasing amounts of trans-unsaturated fats increases the risk of ovulatory infertility [19]. The etiology is likely multifactorial, but prior work has suggested down regulation of PPAR- γ may be a contributing factor [30–32]. PPAR- γ is a nuclear receptor in humans that is primarily responsible for fatty acid storage and glucose metabolism and has been shown to play a role in the ovarian microenvironment [30–32]. Trans fats act to down regulate PPAR- γ and subsequently increase levels of free fatty acids and downstream will increase circulating glucose levels as well [19]. A large body of evidence supports that insulin resistance or insulin insensitivity due to either lifestyle or dietary factors negatively impacts fertility. PPAR- γ agonists have successfully been used to counteract this as treatment in hyperlipidemia and hyperglycemia and have been shown to improve ovulatory dysfunction and oocyte quality in obese females [19, 31, 32]. A diet-induced obesity mouse model demonstrated that embryonic development to the blastocyst stage was significantly improved with the PPAR- γ agonist, rosiglitazone, and was able to overcome and reverse the effects of a high-fat diet at the level of the ovary [31]. Our preliminary findings suggest that a higher erythrocyte index of the commonly consumed trans fat elaidic acid may be associated with impaired embryo development and negative trend in fertilization rate in women undergoing IVF. The potential mechanisms through which this is affected are likely multifold as described above.

There are several major limitations that need to be considered when interpreting these results including the small, heterogeneous sample size and homogenous fatty acid index of patients included. While the sample size was powered to assess embryo development, it was not powered to determine differences in pregnancy, implantation, or live birth rates.

Subgroups of infertility populations were too small to make comparisons within each infertility subgroup, which could have impacted our results. There is also no control for potential confounders including cycle level factors of the associations evaluated. We obtained a single fasting serum sample on cycle day 8 to minimize the potential effect of varying fatty acid concentrations across the menstrual cycle, but results may have further been impacted by gonadotropin stimulation and may not be representative of the patient's baseline. A voluntary dietary survey was given to all subjects, but too few returned the survey to provide meaningful analysis. Although we found no correlation between patient age and BMI with fatty acid levels, these were not included in a regression model and could potentially confound our results. Future studies should include patient BMI, tobacco use, medication use, serum lipid concentrations, dietary intake, and physical activity level to better assess for potential confounding bias. As multiple data points were examined the inherent possibility of type I error should be recognized and our findings should be cautiously interpreted in this context. We corrected for multiple comparisons by establishing a stricter *p* value level of significance by Bonferroni correction, after which, our primary outcome remained significant.

The major strengths of this study are its prospective design and use of a single embryologist blinded to fatty acid index to eliminate possible bias and minimize subjectivity of embryo grade. We used a validated method to determine the 22 fatty acids and the fatty acid index that has been previously described [24]. Our study's preliminary results, taken in the context of limitations as noted above, suggest a negative correlation of the trans fatty acid elaidic acid on embryo development in vitro. We propose larger, future studies control for potential confounding factors such as patient age, BMI, and infertility diagnosis, while also accounting for dietary intake, to better identify novel recommendations that may improve IVF outcomes in a selected patient population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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