Published in final edited form as:
Fertil Steril. 2011 October ; 96(4): 880–883. doi:10.1016/j.fertnstert.2011.07.1115.

Elevated serum alpha-linolenic acid levels are associated with decreased chance of pregnancy after in vitro fertilization

Emily S. Jungheim, MD, MSCI, **George A. Macones, MD, MSCE**, **Randall R. Odem, MD**, **Bruce W. Patterson, PhD**, and **Kelle H. Moley, MD**
Washington University in St. Louis, St. Louis, MO, Department of Obstetrics and Gynecology (ESJ, GAM, RRO, KHM), Department of Internal Medicine Center for Human Nutrition (BWP)

Abstract

Study Objective—To analyze relationships between serum free fatty acid (FFA) concentrations and pregnancy.

Design—Prospective cohort

Setting—University hospital

Patients—91 women undergoing in vitro fertilization (IVF)

Interventions—Serum was analyzed for total and specific serum FFAs including myristic, palmitic, stearic, oleic, linoleic and α-linolenic acids.

Main outcome measures—Univariate analyses were used to identify specific FFAs and other factors associated with pregnancy after IVF. Logistic regression was performed modeling relationships between identified factors and chance of pregnancy.

Results—In unadjusted analyses, women with elevated serum α-linolenic (ALA) levels (highest quartile) demonstrated a decreased chance of pregnancy compared to women with the lowest levels (OR:0.24, 95% CI:0.052–0.792, p=0.022). No associations between other FFAs and pregnancy were identified. In a multivariable regression model, associations between elevated serum ALA levels and decreased chance of pregnancy remained after adjusting for patient age, body mass index, and history of endometriosis or previous live birth (adjusted OR:0.139, 95% CI: 0.028–0.686, p=0.015).

Conclusions—Elevated serum ALA levels are associated with decreased chance of pregnancy in women undergoing IVF. Further work is needed to determine if ALA is involved in early reproductive processes and if the relationship between ALA and pregnancy is associated with excess ALA intake, impaired ALA metabolism or both.

Keywords

free fatty acids; α-linolenic acid; omega 3 fatty acids; in vitro fertilization; embryo implantation; metabolism

Corresponding author: Emily S. Jungheim jungheime@wudosis.wustl.edu Office: 314-286-2400, Fax: 314-286-2455 Washington University 4444 Forest Park Avenue, Suite 3100, Campus Box 8513 St. Louis, MO 63108.

INTRODUCTION

Fatty acids are important substrates in early reproductive events including oocyte maturation(1, 2) and embryo implantation (3). Much attention has been focused on specific free fatty acids (FFA) and their role in the development and prevention of different diseases like heart disease and diabetes, but little is known regarding the effects of specific FFAs on reproduction.

Specimens collected from women undergoing in vitro fertilization allow a unique opportunity to investigate associations between different metabolites like FFAs and various events in the reproductive process. Recently, we performed a prospective cohort study investigating associations between ovarian follicular FFA content and oocyte quality in women undergoing in vitro fertilization (IVF) and found that elevated levels of total follicular FFAs were associated with poor oocyte quality as measured by cumulus oocyte complex (COC) morphology. No associations between specific follicular FFAs and COC morphology were identified nor were any associations between follicular FFAs and pregnancy rates. Correlations between total and specific serum free fatty acids and follicular FFAs were weak raising the possibility that serum FFAs could influence different steps involved in the reproductive process(4). For example, in addition to oocyte maturation FFAs are involved in embryo implantation—as precursors for necessary prostaglandin synthesis, and potentially as ligands for various perioxisome-proliferator-activated receptors (PPAR) involved in the implantation process (3, 5).

Given that serum FFAs may be a source of FFAs for embryo implantation or influence some other step of the reproductive process, we sought to analyze differences in FFA concentrations in serum from women undergoing IVF in regards to pregnancy outcomes.

MATERIALS AND METHODS

Study participants and samples

All study protocols were approved by Washington University's Institutional Review Board. Data for the present investigation were obtained from women originally enrolled into a study of associations between follicular fluid FFAs and cumulus oocyte complex morphology that took place at Washington University in the years 2007–2009(4). All participants underwent a long-agonist controlled ovarian hyperstimulation protocol as previously described (6). Fasting serum was collected from participants the morning of oocyte retrieval and stored at −80° C for batched analysis. Data collected from patient medical charts included: date of birth, body mass index (BMI) (kg/m^2), gravity, parity, tobacco use, and infertility diagnosis. IVF cycle information collected included: total gonadotropin dose, peak estradiol, number of mature ovarian follicles (≥16mm on ultrasound), number of oocytes retrieved, oocyte fertilization method and fertilization rate, number and developmental stage of embryos transferred, number of gestational sacs on initial ultrasound (performed between 5–7 weeks gestational age), and pregnancy outcome.

Women were included in the present study if they were under age 38 and undergoing their first cycle of IVF, and if they had serum drawn at the time of their oocyte retreival. Women were excluded if they had known stage III/IV endometriosis, or if they used sperm obtained from testicular biopsy in their IVF procedure.

Analysis of Serum FFAs

Total and specific serum FFA concentrations were measured as previously described (7). Briefly, C17:0 heptadecanoic acid was added to serum as an internal standard, proteins were

precipitated with ice-cold acetone, and lipids extracted with hexane. The hexane layer was dried down, reconstituted with a buffer, and fatty acid methyl esters (FAMEs) were made using iodomethane. The lipid mixture containing FAMEs was dried down, dissolved in heptane, and injected onto the quantitative GC. The peak areas for the individual FAMEs were quantified relative to the C17:0 methyl ester peak area. A quantitative calibration standard was also run containing all the FAMEs, including C17:0, so that relative peak areas could be converted to absolute amounts of each fatty acid relative to the amount of C17:0 added to the sample.

Specific FFAs measured included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and α-linolenic (18:3) acids.

Measurements and Statistical Analysis

Characteristics, IVF outcomes, and total and specific serum FFA levels were compared from women who achieved pregnancy after IVF (defined as one or more gestational sacs on first trimester ultrasound) to women who did not achieve pregnancy, using appropriate univariable statistics including Student t-test, χ^2 analysis, and Fisher's exact test. Factors identified as significant in the univariable analyses (serum ALA level and endometriosis) or known to be historically associated with pregnancy outcome after IVF (history of prior delivery, age and BMI) were entered into a logistic regression model assessing their association with pregnancy after IVF. Serum ALA levels were re-coded into quartiles for the multivariable model. The lower limit of detection for serum ALA in our assay was 0.0025 μmol/mL and intra-assay variability was <5%.

To investigate whether serum ALA levels might be associated with any specific step of the reproductive process in this study, standard univariable statistics were used to analyze other IVF outcomes in regards to serum ALA levels including ovarian response to gonadotropins (as measured by peak estradiol level, amount of gonadotropin required, number of mature follicles, number of mature oocytes retrieved, and percent good quality oocytes as measured by cumulus oocyte complex morphology), embryo implantation rate, and live birth. All statistical analyses were performed in SPSS, version18 (SPSS Inc., Chicago, IL).

RESULTS

Patient Characteristics

Comparing women who achieved pregnancy after IVF versus women who did not, no differences were detected in their age, BMI, history of previous live birth, number of embryos transferred, or diagnoses including ovulation disorder, fallopian tube abnormality, male factor infertility or unexplained infertility. Women with a history of known endometriosis were found to have a lower chance of pregnancy after IVF (p=0.01) (TABLE 1). Of note, eight of the women who achieved a clinical pregnancy after IVF subsequently experienced spontaneous miscarriages.

Associations Between Serum FFAs and IVF Outcomes

Comparing women who achieved pregnancy (n=57) after IVF to those who did not achieve pregnancy (n=34), no differences in serum total or specific FFA levels were noted except in serum ALA levels (0.00729 μmol/mL ± 0.0065 vs. 0.01 μmol/mL ± 0.0059, p=0.03) (TABLE 2).

Serum ALA levels were divided into quartiles and logistic regression was used to determine the association of these levels with clinical pregnancy after IVF adjusting for age, BMI, history of previous live birth and endometriosis. In our logistic regression analysis, a dose

response relationship was seen between increasing serum ALA levels and decreased odds of clinical pregnancy (TABLE 3). Women in the highest quartile had an 86% lower chance of achieving pregnancy than those with the lowest levels (adjusted odds ratio/AOR 0.14, 95% CI: 0.028–0.69). Women in the third highest quartile had a 79% lower chance of achieving pregnancy (AOR 0.21, 95% CI: 0.05–0.97). Women in the second quartile had a lower chance of achieving pregnancy, but this was not statistically significant (AOR 0.27, 95% CI: 0.055–1.29). In this model endometriosis was also associated with a lower chance of achieving live birth (AOR 0.075, 95% CI: 0.007–0.79). When clinical pregnancy was replaced with live birth in this model, no significant relationship was noted between serum ALA levels and live birth. This is likely attributable to the fact that eight women experienced miscarriages after confirmation of clinical pregnancy by first trimester ultrasound, and none of these women were in the group with the highest ALA levels.

No significant correlation was identified between patients' serum ALA levels and ovarian response to gonadotropin (as measured by amount of gonadotropin required ($r=-0.157$, $p=0.14$) and peak serum estradiol level ($r=0.003$, $p=0.98$)), or percent good quality oocytes as judged by COC morphology ($r=-0.007$, $p=0.95$). A weak negative correlation was detected between serum ALA levels and embryo implantation rates ($r=-0.21$, $p=0.046$).

CONCLUSIONS

In this study of women undergoing IVF, fasting serum ALA levels drawn the morning of oocyte retrieval were negatively associated with embryo implantation rates and occurrence of clinical pregnancy. No associations were seen between serum ALA levels and ovarian response to gonadotropin, oocyte quality as judged by COC morphology, or occurrence of live birth, nor were any associations between other specific serum FFAs or total FFAs and IVF outcomes. Altogether, these findings support a possible role for circulating ALA in embryo implantation. On the other hand, it is possible that the pathways involved in the metabolism of ALA are instead important to embryo implantation, and that circulating levels of ALA merely serve as markers of their own metabolism.

ALA is a short chain omega 3 fatty acid obtained from the diet in foods like flaxseed, soybeans, walnuts, and seed and vegetable oils. It is metabolized into other long chain polyunsaturated fatty acids including eicosapentaneoic acid (EPA) and docosahexaenoic acid (DHA)—both ligands for peroxisome proliferator-activated receptors (PPARs) involved in embryonic and placental development(8). Interestingly, recent evidence suggests PPARs may also be involved in the attachment of endometrial cells to peritoneum and perhaps one mechanism involved in the development of endometriosis (9).

In this study we saw an increased association between elevated circulating ALA levels and the presence of known endometriosis suggesting perhaps that historical associations between endometriosis and decreased implantation rates in IVF and fertility (10) may be attributable either to aberrant PPAR function, or instead aberrant ALA metabolism. Supporting a need for further investigation of ALA metabolism as it relates to both infertility and endometriosis, long-term prospective studies of lifestyle and health outcomes by Chavarro et al and Missmer et al note decreased incidence of both infertility and endometriosis among women with high omega 3 fatty acid intake(11). Furthermore, a recent publication by Pohlmeier et al characterizes reproductive sequelae of over-expression of the n-3 fatty acid desaturase enzyme in a murine model. In this model, authors found that circulating n-3 polyunsaturated fatty acid levels were elevated, and while animals had normal ovulation and fertilization, fewer embryos implanted and there was a high incidence of post-implantation fetal resorption (12). In another recent publication by Ji et al, authors attempting to isolate specific dietary benefits of n-3 fatty acid consumption concluded that “prolonged exposure

to increased concentrations of n-3 fatty acids may be detrimental to reproduction" when their animal model with the highest n-3 fatty acid concentrations failed to produce offspring (13).

There are several limitations to our study that need to be taken into consideration. First, while the findings regarding associations between endometriosis and elevated serum ALA levels are interesting and potentially important, the number of women in the study with known endometriosis was small. There also may have been women in the study with endometriosis who were never diagnosed because not all study participants underwent diagnostic laparoscopy. Further study with larger numbers of patients investigating possible associations between FFAs and the finding of endometriosis are necessary to validate our findings. Also, we do not know if the elevated serum ALA levels were the result of increased ALA consumption or indicative of variations in ALA metabolism or both. This is an area of future research along with study to validate the findings presented in this manuscript as they were obtained through secondary analysis of data collected for other study(4).

Regardless of the etiology of elevated ALA levels in some women, we believe our findings raise questions for future research that are worth exploring. Among many, these questions include whether or not intake of ALA through food and vitamin sources alters serum ALA levels and IVF outcomes, if interventions that alter ALA metabolism would improve pregnancy rates in women with elevated ALA levels after IVF, and finally whether or not ALA levels could be used as a tool to determine how many embryos to transfer back after IVF to optimize pregnancy rates without increasing the risk of multiple gestations. Finally further investigation of a possible relationship between endometriosis and infertility through altered FFA metabolism and PPAR action is necessary as medical interventions may be possible to improve outcomes for affected women thereby preventing the need to resort to surgical treatment to manage pain in the case of endometriosis or IVF for management of infertility.

Acknowledgments

The authors would like to thank Jennifer Shew and Freida Custudio for technical assistance with free fatty acid measurement, the Women and Infant's Health Specimen Consortium at Washington University for assistance with patient enrollment, and the nursing and laboratory staff at Washington University's Center for Reproductive Medicine and Infertility for their assistance in specimen processing.

This work was supported by National Institutes of Health (NIH) Grants K12HD063086-01, UL1RR024992, P30DK056341, and L50HD062021-01.

References

1. Downs SM, Mosey JL, Klinger J. Fatty acid oxidation and meiotic resumption in mouse oocytes. Mol Reprod Dev. 2009; 76:844–53. [PubMed: 19455666]
2. Sturmey RG, Reis A, Leese HJ, McEvoy TG. Role of fatty acids in energy provision during oocyte maturation and early embryo development. Reprod Domest Anim. 2009; 44(Suppl 3):50–8. [PubMed: 19660080]
3. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med. 2001; 345:1400–8. [PubMed: 11794174]
4. Jungheim ES, Macones GA, Odem RR, Patterson BW, Lanzendorf SE, Ratts VS, et al. Associations between free fatty acids, cumulus oocyte complex morphology and ovarian function during in vitro fertilization. Fertil Steril. 2011; 95:1970–4. [PubMed: 21353671]
5. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002; 53:409–35. [PubMed: 11818483]

6. Jungheim ES, Lanzendorf SE, Odem RR, Moley KH, Chang AS, Ratts VS. Morbid obesity is associated with lower clinical pregnancy rates after in vitro fertilization in women with polycystic ovary syndrome. Fertil Steril. 2009; 92:256–61. [PubMed: 18692801]

7. Patterson BW, Zhao G, Elias N, Hachey DL, Klein S. Validation of a new procedure to determine plasma fatty acid concentration and isotopic enrichment. J Lipid Res. 1999; 40:2118–24. [PubMed: 10553015]

8. Jawerbaum A, Capobianco E. Review: Effects of PPAR activation in the placenta and the fetus: implications in maternal diabetes. Placenta. 2011; 32(Suppl 2):S212–7. [PubMed: 21194748]

9. Kavoussi SK, Witz CA, Binkley PA, Nair AS, Lebovic DI. Peroxisome-proliferator activator receptor-gamma activation decreases attachment of endometrial cells to peritoneal mesothelial cells in an in vitro model of the early endometriotic lesion. Mol Hum Reprod. 2009; 15:687–92. [PubMed: 19643817]

10. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst Rev. 2006:CD004635. [PubMed: 16437491]

11. Missmer SA, Chavarro JE, Malspeis S, Bertone-Johnson ER, Hornstein MD, Spiegelman D, et al. A prospective study of dietary fat consumption and endometriosis risk. Hum Reprod. 2010; 25:1528–35. [PubMed: 20332166]

12. Pohlmeier WE, Hovey RC, Van Eenennaam AL. Reproductive abnormalities in mice expressing omega-3 fatty acid desaturase in their mammary glands. Transgenic Res. 2011; 20:283–92. [PubMed: 20532624]

13. Ji S, Hardy RW, Wood PA. Transgenic expression of n-3 fatty acid desaturase (fat-1) in C57/BL6 mice: Effects on glucose homeostasis and body weight. J Cell Biochem. 2009; 107:809–17. [PubMed: 19396841]

TABLE 1

Demographic and clinical characteristics of all study participants

	Pregnant N = 57	Not pregnant N = 34	P-value
Age (years)	32.9 ± 3.3	32.1 ± 3.7	0.25
BMI (kg/m^2)	25.7 ± 5.6	24.6 ± 3.8	0.3
Previous live birth	15	4	0.09
History of known endometriosis	1	6	0.01
Ovulation disorder	11	6	0.84
Fallopian tube abnormality	12	8	0.78
Male factor infertility	18	14	0.38
Unexplained infertility	14	5	0.26
Number of embryos transferred	2.2 ± 0.6	2.3 ± 0.6	0.56

TABLE 2

Results of univariate analyses comparing total and specific FFA levels in women who were pregnant afterIVF vs. women who were not pregnant

Serum free fatty acid (μmol/ml)	Pregnant N = 57	Not pregnant N = 34	P-value
Total free fatty acid	0.57 ± 0.17	0.60 ± 0.19	0.38
Myristic acid	0.01 ± 0.005	0.01 ± 0.005	0.69
Palmitic acid	0.14 ± 0.047	0.15 ± 0.05	0.46
Stearic acid	0.05 ± 0.015	0.05 ± 0.016	0.38
Oleic acid	0.21 ± 0.07	0.23 ± 0.075	0.47
Linoleic acid	0.12 ± 0.04	0.13 ± 0.047	0.34
α-linolenic acid	0.00729 ± 0.0065	0.01 ± 0.0059	0.032

TABLE 3

Multivariable logistic regression: Serum ALA level and chance of pregnancy

Factor	Unadjusted RR	Adjusted OR†	95% CI†	P†
Tertile 1 serum ALA (0–0.0025 μmol/ml)	1.15	1		
Tertile 2 serum ALA (0.0026–0.009 μmol/ml)	0.91	0.3	0.01–1.29	0.10
Tertile 3 serum ALA (0.01–0.013 μmol/ml)	1.03	0.2	0.044–0.97	0.046
Tertile 4 serum ALA (0.014–0.03 μmol/ml)	0.81	0.14	0.03–0.69	0.02

†Model adjusted for age, BMI, history of known endometriosis and history of previous live birth