Published in final edited form as:
Fertil Steril. 2011 May ; 95(6): 1970–1974. doi:10.1016/j.fertnstert.2011.01.154.

Associations between free fatty acids, cumulus oocyte complex morphology and ovarian function during in vitro fertilization

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

Abstract

Objective—To determine if follicular free fatty acid (FFA) levels are associated with cumulus oocyte complex morphology

Design—Prospective cohort study

Setting—University in vitro fertilization (IVF) practice

Patients—102 women undergoing IVF

Interventions—Measurement of FFAs in serum and ovarian follicular fluid

Main Outcome Measures—Total and specific follicular and serum FFA levels, correlations between follicular and serum FFAs, and associations between follicular FFA levels and markers of oocyte quality including cumulus oocyte complex (COC) morphology

Results—Predominant follicular fluid and serum FFAs were oleic, palmitic, linoleic and stearic acids. Correlations between follicular and serum FFA concentrations were weak (r=0.252, 0.288, 0.236, 0.309 respectively for specific FFAs; r=0.212 for total FFAs). A receiver operator characteristic curve determined total follicular FFAs ≥ 0.232 μmol/ml distinguished women with lower versus higher percentage COCs with favorable morphology. Women with elevated follicular FFAs (n=31) were more likely to have COCs with poor morphology than others (n=71) (OR 3.3, 95% CI:1.2–9.2). This relationship held after adjusting for potential confounders including age, BMI, endometriosis and amount of gonadotropin administered (β=1.2; OR 3.4, 95% CI:1.1–10.4).

Conclusions—Elevated follicular FFA levels are associated with poor COC morphology. Further work is needed to determine what factors influence follicular FFA levels and if these factors impact fertility.

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.

Keywords

oocyte; cumulus oocyte complex; ovarian follicle; free fatty acids

INTRODUCTION

Serum FFAs are elevated in obesity and PCOS (1)—two common conditions in reproductive age women and both associated with anovulation, decreased fertility and suboptimal reproductive outcomes(2). Elevated serum FFAs are also associated with development of type 2 diabetes (3), possibly by increasing insulin resistance in skeletal muscle (4) and/or inducing apoptosis in pancreatic beta cells(5). FFAs may have similar adverse effects on ovarian follicles and developing oocytes.

Data from animal studies shows oocytes metabolize FFAs(6,7). Palmitic, stearic, and oleic acids are the predominant FFAs in bovine ovarian follicles(8). In these animals, elevated follicular palmitic and stearic acids are associated with impaired oocyte maturation and fertilization rates, and poor quality embryos (8) suggesting FFA have direct, adverse effects on oocytes. Studies also demonstrate excess palmitic acid induces apoptosis in human and bovine granulosa cells and impairs their ability for steroidogenesis—a critical function of granulosa cells in supporting ovarian follicular development and oocyte maturation(9,10).While animal data suggests FFAs affect ovarian function, little is known relating FFAs to human reproductive potential. Given this knowledge gap, we sought to determine the predominant FFAs in the human ovarian follicle with samples collected from women undergoing IVF. Using results obtained from these samples we asked whether elevations in FFAs were associated with markers of reduced oocyte quality and suboptimal ovarian response to gonadotropin stimulation. Finally, we sought to determine if obesity and PCOS were associated with increased levels of total or specific FFAs in ovarian follicular fluid.

MATERIALS AND METHODS

Patient characteristics and samples

Washington University's Institutional Review Board approved all study protocols. Women were enrolled the morning of oocyte retrieval after undergoing controlled ovarian hyperstimulation (COH) with injected gonadotropins as previously described(11). Inclusion criteria were as follows: first IVF cycle, and age <38 years. Women were excluded if they had not undergone our standard COH protocol, if they had known stage III/IV endometriosis, or if sperm used in their procedures was obtained from testicular biopsy as these factors could influence study outcomes(12–14).Data collected from medical charts included age, body mass index (BMI) (kg/m^2), gravity, parity, tobacco use, and infertility diagnosis.

Participants were fasting the morning of sample collection. Serum was extracted from venous blood.. Ovarian follicular fluid was collected as previously described(15). Briefly, each ovary was evaluated for an accessible follicle of mature size (≥16mm). The chosen follicle was punctured and aspirated with a 16-gauge needle under ultrasound-guidance. Oocytes were isolated from follicular fluid for evaluation and culture. Remaining follicular fluid was centrifuged to remove residual cells, and stored with serum samples at −80°C for batched analysis. Study follicular fluid was always obtained from the first follicular aspirate from a given ovary, and was collected from both ovaries in most patients. If no oocyte was isolated from the first aspirated follicle from at least one ovary, the patient was excluded from further study.

Analysis of serum and ovarian follicular fluid FFAs

Total and specific serum and follicular FFA concentrations were quantified as previously described(16). Specific FFAs analyzed 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.

IVF cycle characteristics and outcomes

The following cycle data was collected: total gonadotropin dose, peak estradiol, number of mature ovarian follicles, number of oocytes retrieved, oocyte insemination method and fertilization rate, grade of cumulus oocyte complex (COC) obtained from initial follicular aspirate, percent grade 3 or 4 COCs, number and developmental stage (day 3 vs. day 5) of embryos transferred, number of gestational sacs on ultrasound, and pregnancy outcome.

Oocyte fertilization rate as was defined as number of oocytes fertilized divided by number of oocytes inseminated. Blinded embryologists determined COC grade—a surrogate marker of oocyte quality based on association between cumulus cell complex dispersion and oocyte maturity previously described by Veeck et al (TABLE 1)(17).

Measurements and statistical analysis

Sample size was aimed at determining if elevated total follicular FFAs were associated with poor oocyte quality, and it was based on the following assumptions: 0.05 type I error, 0.2 type II error, 60% probability of good COC quality in women with lower total follicular FFA levels and 30% probability of good COC quality in others at a 1:1 case:control ratio. 96 women were enrolled to meet these assumptions. 6 additional women were enrolled in anticipation of difficulties processing patient specimens.

Correlation between total and specific FFAs in serum and follicular fluid was measured by Pearson's or Spearman's correlation coefficients as appropriate. In women with follicular fluid collected from a follicle on both the right and left ovaries, correlation between samples was measured to determine if fluid from one follicle was likely representative of another, un-sampled follicle. A receiver operator characteristic (ROC) curve was established to determine what total follicular FFA concentration best predicted reduced COC quality (defined as <60% grade 3 or 4 COCs). Once this concentration was established, patients were allocated to one of two groups, those with lower total follicular FFAs (< 0.232 μmol/ml) or those with higher total follicular FFAs (≥ 0.232 μmol/ml). The resulting groups were compared for IVF cycle outcomes using standard bivariate statistics. Comparisons yielding p-values of < 0.05 were considered significant. Relationships between follicular FFA concentration and COC grade were modeled with logistic regression adjusting for age, BMI, diagnosis of endometriosis, and total amount of gonadotropin administered. Relationships between elevated follicular FFA levels and percentage of good quality COCs were modeled with linear regression adjusting for the same factors. We also investigated total and specific serum and follicular FFA concentrations in overweight vs. normal weight patients and in women with and without PCOS as defined by 2003 Rotterdam criteria(18). Analyses were performed in SPSS version 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient characteristics

102 women were enrolled. Average patient age was 32.7±3.5 years. Average BMI was 25.38±5.1 kg/m^2. 41 subjects were overweight, 23 were parous, and 6 used tobacco. Infertility diagnoses included PCOS (n=6), other ovulation disorder (n=13), fallopian tube abnormalities (n=20), endometriosis (n=8), and male factor infertility (n=35). Several participants had multiple diagnoses, and 22 had unexplained infertility.

FFAs in serum and ovarian follicular fluid

Follicular fluid was available for 102 patients and serum for 93. Overall serum FFA content for participants was as follows: 38% oleic, 24% palmitic, 21% linoleic, and 10% stearic acids. Follicular fluid FFA content was as follows: 31% oleic, 27% palmtic, 25% linoleic, and 12% stearic acids. Correlation analysis revealed strong relationships between total and specific FFAs in follicular fluid obtained from two different ovaries within the same woman (correlation coefficient range 0.6–0.85 and $p<0.0001$ for total and all specific FFAs). Conversely, only weak correlations were demonstrated between concentrations of total and specific FFAs in serum and follicular fluid (TABLE 2).

Associations between total follicular FFAs and COC morphology and ovarian function as measured by IVF cycle outcomes

Applying results of our ROC curve (AUC 0.693), 71 women were determined to have lower total follicular FFA concentrations (<0.232 μmol/ml) and 31 women had higher total follicular FFAs (≥0.232 μmol/ml) (75% sensitive, 71% specific). No differences were noted between the groups for age, BMI, or method of oocyte insemination. Interestingly, more women diagnosed with endometriosis were found among women with elevated follicular FFAs than those with lower levels(6/31 vs. 2/71; p=0.004; OR 8.28, 95% CI: 1.37–64.01) (TABLE 3).

IVF outcomes did not differ between groups for amount of gonadotropin administered, number of mature follicles, number of oocytes retrieved, oocyte fertilization rate, or for clinical pregnancy or delivery rates. On the other hand, the odds of a patient having a poor quality COC retrieved from her first aspirated follicle was higher if the associated total follicular FFA level was ≥0.232 μmol/ml (3.28, 95% CI:1.05–10.36). This relationship held after adjusting for potential confounders (β=1.2; OR 3.4, 95%CI:1.2–9.2). Percentage of grade 3 or 4 COCs was also significantly lower in women with follicular FFA concentrations (≥0.232 μmol/ml) compared to other women (69.86% vs. 78.23%; p=0.041). This association also held after adjusting for potential confounders (Model: R^2=0.12, p=0.031; β= −0.229, 95% CI: −0.158 to −0.010, p=0.027).

There was a trend toward decreased number of cleavage stage embryos (post-fertilization day 3) in women with elevated follicular FFA concentrations versus women with lower levels (6.77 vs. 8.55; p=0.05). Accordingly there was also an increased trend toward transfer of embryos on day 3 after fertilization among women with elevated follicular FFAs (23/31 vs. 40/71; p=0.088) rather than pushing these women for transfer of embryos on day 5—a practice our lab reserves for patients with a greater quantity of good quality embryos. Finally, there was a trend toward decreased implantation of embryos in women with elevated follicular FFAs (0.428 vs. 0.285; p=0.085) (TABLE 4).

Associations between overweight and PCOS with FFA content of serum and follicular fluid

BMI correlated weakly with serum palmitic acid concentration (R=0.221; p=0.043). No other significant correlations between BMI and total or specific serum or follicular FFAs were noted, however dichotomizing patients into overweight (BMI ≥25 kg/m^2, n=52) vs. normal weight (BMI <25 kg/m^2, n=37) revealed overweight women had higher serum palmitic and linoleic acid levels than thinner women (0.16 vs. 0.14 μmol/ml; p = 0.03; 0.14 vs. 0.12 μmol/ml; p = 0.02 respectively). There were no differences in follicular FFAs noted between overweight vs. normal weight patients. No significant differences were noted in women with PCOS vs. those without PCOS, but numbers of women with PCOS in this study were small (n=6).

DISCUSSION

Our findings demonstrate that predominant FFAs in the ovarian follicle include oleic, palmitic, stearic, and linoleic acids—the latter being an essential FFA obtained from the diet whereas the others are synthesized by the body. The predominant FFAs in the ovarian follicle were consistent with those found in serum, however, concentrations of FFAs in follicular fluid had only a weak correlation with FFAs in serum. These results suggest cells of the ovarian follicle metabolize specific FFAs at different rates, or perhaps certain FFAs are transported preferentially into the ovarian follicle. In any case, we also found associations between elevations in total follicular FFAs and poorer COC quality further suggesting excess FFAs adversely influence ovarian follicular function.

Glucose is a better studied energy source for oocytes than FFAs(19). In conditions of excess circulating glucose like type I diabetes, ovarian follicular function is adversely affected with increased granulosa cell apoptosis, abnormal ovarian follicular development, and maturational delay and mitochondrial abnormalities in oocytes(19–21). Similar to glucose, it is possible at normal levels FFAs are utilized by the oocyte and ovarian follicle without consequence, but that in excess FFAs are detrimental. We theorize this could occur through direct effects on fatty acid metabolism key to oocyte maturation (6) via effects on PPARγ, an enzyme thought to be important in oocyte development and ovarian function (22) for which FFAs can act as a ligand (23), or alternatively via inhibitory effects on lipogenic pathways that may be important to granulosa cell function(24). Interestingly, PPARγ activators are effective in inducing ovulation in anovulatory women with PCOS(25,26).

What conditions affect FFA concentrations and composition within serum and ovarian follicular fluid are not clear. Increasing BMI is associated with anovulation and subfertility (2), and as mentioned, previous studies of obesity and PCOS have identified associations between these conditions and elevated serum FFAs (1). We did not detect differences in follicular FFAs between overweight and normal weight women, however, overweight women in our sample had multiple different indications for undergoing IVF with only 5 of the 41 overweight women having the diagnosis of PCOS or other ovulatory dysfunction. We also did not detect differences in follicular FFAs in women with and without PCOS although only 6 women with PCOS participated in our study. Perhaps with a larger sample of PCOS patients or anovulatory obese women would reveal associations between FFAs and these conditions..

While adiposity may contribute to serum and follicular fluid FFA concentrations, diet may also be important. Previous work with Nurses' Health Study II (NHSII) data has shown that increased intake of *trans*-unsaturated fats like those found in commercially fried and baked products increases the risk of ovulatory infertility(27). Our assay for measuring FFAs in the serum and follicular fluid did not distinguish between the *trans* and *cis* configurations of FFAs, but future work relating dietary fatty acid intake to oocyte quality and IVF outcomes is worth exploring. NHSII data has also revealed associations between increased *trans*-unsaturated fat intake and the diagnosis of endometriosis(28). Interestingly, more women with known endometriosis were in our group of women with elevated follicular FFAs. We excluded women with known stage III/IV endometriosis from our study as endometriosis can affect oocyte quality (12), but our findings raise the possibility that it is not endometriosis that affects oocyte quality, but instead some aberration in FFA metabolism or dietary intake of FFAs. If this is the case, we suspect women with infertility related to endometriosis could benefit from treatment with PPARγ activators as suggested by others (29,30) or perhaps with modifications in types of fat consumed.

The major limitation of our study is the subjective and controversial nature of our chosen method for assessing oocyte quality(17). We chose to evaluate COC morphology as a surrogate measure of oocyte quality as this method is noninvasive, and it is logistically feasible in a clinical IVF laboratory setting. On the other hand, oocyte maturity and COC morphology can be disparate, and there is a lack of data demonstrating association between COC morphology and reproductive potential. Embryo grading is also subjective, however, there are more standardized criteria available for grading embryos, and there is also data available linking embryo quality to reproductive outcomes. We chose not to use embryo quality as an outcome measure in this study for several reasons. The first and most important being that our lab routinely pools fertilized eggs making it impossible to link a specific embryo to the oocyte or follicle contents it was derived from. The second reason is that embryos are not graded until they have been in culture for some time. Time in culture could influence embryo quality in a positive or negative way and introduce unmeasurable confounding(2). We were reassured in our conclusions that elevated follicular FFA levels are associated with poor oocyte quality by finding trends in surrogate markers of oocyte quality suggesting the same association. These surrogate markers include a decreased number of cleavage stage embryos ($p=0.05$), an increase in day 3 transfers ($p=0.09$), and a trend toward decreased implantation of embryos ($p=0.09$) in women with elevated follicular FFAs. Further study with a larger number of patients may reveal significant differences in these outcomes and allow for validation of our study's findings.

The major strengths of our study are its prospective design and the fact that its findings are supported by other clinical and basic science studies demonstrating associations between FFAs and female reproduction (1,6–9,27,28,31–33). We propose future study of factors known to adversely affect FFA metabolism and their associations with reproductive function to identify novel treatments that may improve fertility in affected women.

Acknowledgments

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

The authors would like to thank Dr. David Alpers for his review of this manuscript, participating patients, Jennifer Shew and Freida Custudio for technical assistance with free fatty acid measurement, the Women'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.

REFERENCES

1. Holte J, Bergh T, Berne C, Lithell H. Serum lipoprotein lipid profile in women with the polycystic ovary syndrome: relation to anthropometric, endocrine and metabolic variables. Clin Endocrinol (Oxf). 1994; 41:463–471. [PubMed: 7955457]
2. Jungheim ES, Moley KH. Current knowledge of obesity's effects in the pre- and periconceptional periods, and avenues for future research. American Journal of Obstetrics and Gynecology. 2010
3. Pankow JS, Duncan BB, Schmidt MI, Ballantyne CM, Couper DJ, Hoogeveen RC, et al. Fasting plasma free fatty acids and risk of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes Care. 2004; 27:77–82. [PubMed: 14693970]
4. Boden G. Obesity and free fatty acids. Endocrinol Metab Clin North Am. 2008; 37:635–646. viii–ix. [PubMed: 18775356]
5. Lai E, Bikopoulos G, Wheeler MB, Rozakis-Adcock M, Volchuk A. Differential activation of ER stress and apoptosis in response to chronically elevated free fatty acids in pancreatic beta-cells. American journal of physiology. 2008; 294:E540–E550. [PubMed: 18198352]
6. Downs SM, Mosey JL, Klinger J. Fatty acid oxidation and meiotic resumption in mouse oocytes. Mol Reprod Dev. 2009; 76:844–853. [PubMed: 19455666]

7. 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 3:50–58. [PubMed: 19660080]
8. Leroy JL, Vanholder T, Mateusen B, Christophe A, Opsomer G, de Kruif A, et al. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction (Cambridge, England). 2005; 130:485–495.
9. Mu YM, Yanase T, Nishi Y, Tanaka A, Saito M, Jin CH, et al. Saturated FFAs, palmitic acid and stearic acid, induce apoptosis in human granulosa cells. Endocrinology. 2001; 142:3590–3597. [PubMed: 11459807]
10. Vanholder T, Leroy JL, Soom AV, Opsomer G, Maes D, Coryn M, et al. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Animal reproduction science. 2005; 87:33–44. [PubMed: 15885439]
11. 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; 92:256–261. [PubMed: 18692801]
12. Garrido N, Navarro J, Remohi J, Simon C, Pellicer A. Follicular hormonal environment and embryo quality in women with endometriosis. Hum Reprod Update. 2000; 6:67–74. [PubMed: 10711831]
13. Liu J, Tsai YL, Katz E, Compton G, Garcia JE, Baramki TA. Outcome of in-vitro culture of fresh and frozen-thawed human testicular spermatozoa. Hum Reprod. 1997; 12:1667–1672. [PubMed: 9308791]
14. Arslan M, Bocca S, Mirkin S, Barroso G, Stadtmauer L, Oehninger S. Controlled ovarian hyperstimulation protocols for in vitro fertilization: two decades of experience after the birth of Elizabeth Carr. Fertil Steril. 2005; 84:555–569. [PubMed: 16169382]
15. Riley-Vargas RC, Lanzendorf S, Atkinson JP. Targeted and restricted complement activation on acrosome-reacted spermatozoa. J Clin Invest. 2005; 115:1241–1249. [PubMed: 15849610]
16. 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; 40:2118–2124. [PubMed: 10553015]
17. Veeck LL. Oocyte assessment and biological performance. Ann N Y Acad Sci. 1988; 541:259–274. [PubMed: 3195909]
18. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81:19–25.
19. Jungheim ES, Moley KH. The impact of type 1 and type 2 diabetes mellitus on the oocyte and the preimplantation embryo. Semin Reprod Med. 2008; 26:186–195. [PubMed: 18302110]
20. Chang AS, Dale AN, Moley KH. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. Endocrinology. 2005; 146:2445–2453. [PubMed: 15718275]
21. Wang Q, Ratchford AM, Chi MM, Schoeller E, Frolova A, Schedl T, et al. Maternal diabetes causes mitochondrial dysfunction and meiotic defects in murine oocytes. Mol Endocrinol. 2009; 23:1603–1612. [PubMed: 19574447]
22. Minge CE, Bennett BD, Norman RJ, Robker RL. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. Endocrinology. 2008; 149:2646–2656. [PubMed: 18276752]
23. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002; 53:409–435. [PubMed: 11818483]
24. Robker RL, Akison LK, Bennett BD, Thrupp PN, Chura LR, Russell DL, et al. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. J Clin Endocrinol Metab. 2009; 94:1533–1540. [PubMed: 19223519]
25. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. CD003053.

26. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab. 1996; 81:3299–3306. [PubMed: 8784087]
27. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. Dietary fatty acid intakes and the risk of ovulatory infertility. Am J Clin Nutr. 2007; 85:231–237. [PubMed: 17209201]
28. 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. 25:1528–1535. [PubMed: 20332166]
29. Moravek MB, Ward EA, Lebovic DI. Thiazolidinediones as therapy for endometriosis: a case series. Gynecol Obstet Invest. 2009; 68:167–170. [PubMed: 19641325]
30. Lebovic DI, Mwenda JM, Chai DC, Mueller MD, Santi A, Fisseha S, et al. PPAR-gamma receptor ligand induces regression of endometrial explants in baboons: a prospective, randomized, placebo- and drug-controlled study. Fertil Steril. 2007; 88:1108–1119. [PubMed: 17498714]
31. Wonnacott KE, Kwong WY, Hughes J, Salter AM, Lea RG, Garnsworthy PC, et al. Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. Reproduction. 139:57–69. [PubMed: 19789173]
32. Ferguson EM, Leese HJ. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Mol Reprod Dev. 2006; 73:1195–1201. [PubMed: 16804881]
33. Jungheim ES, Schoeller EL, Marquard KL, Louden ED, Schaffer JE, Moley KH. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. Endocrinology. 151:4039–4046. [PubMed: 20573727]

TABLE 1

Oocyte grading criteria based on cumulus cell complex (CCC) dispersion

Poor Quality	Grade 1	Post-mature, CCC not dispersed
	Grade 2	Germinal vesicle, CCC not dispersed
Good Quality	Grade 3	Homogeneous granularity, CCC somewhat expanded & dispersed
	Grade 4	CCC expanded, clear cumulus & oocyte

TABLE 2

Correlation between serum and follicular free fatty acids

Free fatty acid	Mean serum vs. follicular fluid FFA concentration (μmol/ml)	R	p-value
Total free fatty acids	0.58 ± 0.18 vs. 0.204 ± 0.06	0.212	0.042
Palmitic acid	0.15 ± 0.05 vs. 0.056 ± 0.02	0.288	0.005
Stearic acid	0.05 ± 0.016 vs. 0.0239 ± 0.009	0.309	0.003
Oleic Acid	0.22 ± 0.07 vs. 0.064 ± 0.02	0.252	0.015
Linoleic acid	0.13 ± 0.04 vs. 0.05 ± 0.02	0.236	0.023

TABLE 3

Characteristics of women with elevated follicular FFAs vs. women with lower FFAs undergoing IVF

	Total follicular free fatty acid concentration < 0.232 μmol/ml (n=71)	**Total follicular free fatty acid concentration ≥ 0.232 μmol/ml (n=31)**	**p-value**
Age	32.62 (3.5)	32.77 (3.6)	0.84
BMI	25.44 (5.3)	25.14 (4.4)	0.78
PCOS	4 (6%)	2 (6%)	0.87
Other ovulation disorder	10 (14%)	3 (10%)	0.75
Known endometriosis	**2 (3%)**	**6 (19%)**	**0.009 (OR 8.28, 95% CI: 1.37–64.01)**
Tubal factor infertility	15 (21%)	5 (16%)	0.55
Male factor infertility	22 (31%)	9 (29%)	0.43
Unexplained infertility	17 (24%)	5 (16%)	0.38
Use of conventional insemination	13 (18%)	5 (16%)	0.79

TABLE 4

IVF outcomes in women with elevated vs. lower follicular FFAs

	Total follicular free fatty acid concentration < 0.232 μmol/ml (n=71)	Total follicular free fatty acid concentration ≥ 0.232 μmol/ml (n=31)	p-value	OR (95% CI)
Total gonadotropin required (IU)	1929.23 (±858.3)	2101.21 (±909.4)	0.36	
Peak E2 (pg/ml)	2112.49 (±928.6)	2144 (±1166.1)	0.88	
Mature follicles on ultrasound	5.6 (±2.8)	5.5 (±2.5)	0.90	
Number of oocytes collected	14.38 (±6.6)	12.19 (±5.3)	0.11	
Poor quality oocyte collected from first follicle punctured	**9 (13%)**	**10 (32%)**	**0.027**	**3.3 (1.2–9.2)**
Percent good quality oocytes	**78.2% (±15.1)**	**69.9% (±19.8)**	**0.04**	
Fertilization rate	64.6% (±17.2)	61.9% (±23.1)	0.52	
Number of cleavage stage embryos	8.55 (±4.4)	6.77 (±3.52)	0.05	
Day 3 transfer	40 (56%)	23 (74%)	0.09	2.28 (0.88–5.65)
Day 5 transfer	32 (45%)	8 (26%)	0.07	0.42 (0.17–1.08)
Embryo implantation rate	42.8% (±39.0)	28.5% (±32.8)	0.09	
Clinical pregnancies	45 (63%)	16 (52%)	0.27	0.62 (0.26–1.45)
Live birth	37 (52%)	15 (48%)	0.73	0.86 (0.37–2.01)