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Association between blood lipid level and embryo quality during in vitro fertilization

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Abstract

The aim of this study was to investigate the relationship between blood lipid level and the parameters of embryo morphology of in vitro fertilization (IVF).

A total of 488 patients undergoing conventional IVF were divided into pregnant (n = 286) and nonpregnant (n = 202) groups. Levels of triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoprotein (LDL), lipoprotein (a), lipoprotein (b), and embryo outcomes were studied. Spearman correlation was performed to analyze the correlation between blood lipid levels and embryo quality in pregnant group.

The normal fertilization rate and number of good quality embryos were higher than nonpregnant group ($P < .05$). TG, TC, and LDL levels were negatively correlated with number of normal fertilized oocytes, while TG, TC, and Lp(b) were negatively correlated with number of good quality embryos. TG level was negatively correlated with number of oocytes and cleavage embryos while HDL and Lp(a) were positively correlated with number of oocytes, normal fertilized oocytes and cleavage embryos ($P < .05$).

TG, TC, LDL, and Lp(b) levels had negative correlation with embryo quality, while HDL and Lp(a) had positive correlation with the embryo quality. Our present findings showed blood lipid levels may provide certain reference for the prediction of IVF pregnancy outcome.

Abbreviations: BMI = body mass index, FSH = follicle-stimulating hormone, hCG = human chorionic gonadotropin, HDL = high-density lipoproteins, IVF = in vitro fertilization, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglycerides.

Keywords: blood lipid level, embryo quality, infertility

1. Introduction

Female infertility was the inability to conceive after 2 years of regular intercourse in a woman after 1 year of unprotected intercourse.[1,2] Many evidences suggest that dyslipidemia is a major determinant in the progression of infertility.[3,4] Evidence from human in vitro fertilization (IVF) studies indicate that an abnormal maternal serum lipid profile is associated with poorer oocyte quality, ovarian function and embryo development, indicating a potential reduction in fecundity.[5] Studies have demonstrated that treatments of metabolic disorders are absolutely necessary in addition to rebuilding their menstrual cycles and recovering ovulation.[6,7] Additionally, Shalaby et al found that male rats fed on a high-cholesterol diet showed a significantly decreased fertility rate compared with those fed on a cholesterol-free diet,[8] whereas anticholesterol therapy would dramatically improve this condition.

Dyslipidemia is defined as the elevation of plasma cholesterol, triglycerides (TGs), or both, or a low level of high-density lipoprotein (HDL).[9] HDL seems to be the main components in follicular fluid during human follicular formation.[10] Victor et al demonstrated a negative correlationship between follicular fluid HDL cholesterol and the fragmentation rate of embryos after 72 hours of IVF culture in vitro.[11] Additionally, a large number of animal experiments suggest that HDL metabolism exhibits an important effect in embryonic development.[12,13] However, the association between other lipid parameters such as TG, low-density lipoprotein (LDL), lipoprotein (a), lipoprotein (b), and the embryo quality were rarely reported.

Therefore, the present study was designed to comprehensively study the correlation between the blood lipid levels, including TG, TC, HDL, LDL, Lp(a) and Lp(b), and the embryo quality. This paper may provide certain reference for the treatment of IVF.

2. Methods

2.1. Patients

We retrospectively collected the clinical data of patients undergoing conventional IVF treatment at the Reproductive

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SW and JW contributed equally to this paper and thus shared the co-first authorship.

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Medicine Center of the Affiliated Drum Tower Hospital of Nanjing University Medical between August 2016 and August 2017. The inclusion criteria are as follows:

(1) females aged ≤36 years old,
(2) patients underwent the first IVF cycle,
(3) patients whose serum basal follicle-stimulating hormone (FSH) not greater than 10 IU/mL,
(4) patients with tubal factor infertility; and
(5) patients underwent 2 embryos transfer at Day 3.

The exclusion criteria are as follows:

(1) women with abnormal endocrine hormones,
(2) oocyte donation cycles,
(3) nonejaculated sperm (fine needle aspiration, testicular sperm aspiration, microepididymal sperm aspiration, percutaneous epididymal sperm aspiration); and
(4) aneuploidy or a specific disease by preimplantation genetic screening. This study was approved by the Ethics Committee of Affiliated Drum Tower Hospital of Nanjing University Medical School.

2.2. Measurements of the level of blood lipid and hormone

Fasting blood samples were collected in heparinized evacuated tubes (Vacutainer; BD). Serum samples were obtained by ultracentrifugation of plasma. Standardized enzymatic procedures were used for the measurement of plasma levels of TG, TC, HDL, LDL, Lp(a) and Lp(b) by using a Hitachi 704 automated spectrophotometer (Hitachi; Tokyo, Japan). Enzymatic assays were used to determine levels of TG and TC (GPO-PAP). HDL was measured using enzymatic-chemical modification and LDL-C was measured using selective solubilization. Lp(a) and Lp(b) were determined by nephelometric assay.

2.3. Ovarian stimulation

Recombinant FSH (Gonal-F, Serono Switzerland) was used for controlled ovarian hyperstimulation after pituitary down-regulation using GnRH agonist (triptorelin; Ferring Pharmaceuticals, Germany) initiated on Day 21 of the cycle as described previously.[11] Doses of rFSH (150–250 IU/d) were adjusted by estradiol levels and follicular growth. Once 3 or more follicles reached a diameter of >16 mm and the diameter of leading follicle reached ≥18 mm, final oocyte maturation was induced by 5000 to 10,000 IU of human chorionic gonadotropin (hCG, Ferring Pharmaceuticals). Oocyte retrieval was performed 36 hours after hCG was injected through the transvaginal route with an ultrasound guidance under local anesthesia.

2.4. Embryo culture and transfer

Embryo culture was carried out using sequential culture medium (Gseries, Vitrolife, Sweden). Fertilization on Day 0 was performed in G-IVF^{TM} (Vitrolife) under the condition of mineral oil (Vitrolife). In the next morning on Day 1, the oocytes were placed in microdrops (50 μL) of G-1^{TM} (Vitrolife) respectively under the condition of mineral oil and cultured in incubators at 37°C containing 89% N_2, 6% CO_2, and 5% O_2. Then, an inverted microscope equipped with Hoffman modulation contrast (Olympus, Japan) was used to assess the oocytes. Fertilization was checked at 16 to 20 hours post-insemination (Day 1). Embryos with 2PN or 1PN were considered as normal fertilization. Then embryos were observed at 44 ± 1 hour post-insemination (Day 2) and 68 ± 1 hour post-insemination (Day 3) to evaluate the cleavage and embryos quality. They were evaluated using the consensus scoring system[14] including the blastomere numbers, fragmentation rate, and blastomeres symmetry. Good quality embryos were

(1) [2PN] on Day 1,
(2) [4] cells on Day 2,
(3) [7] to 12 cells on Day 3,
(4) no severe uneven size, and
(5) less than 15% fragmentation.

Two embryos with the best quality were transferred into the uterus.

The serum or urinary concentration of β-HCG was measured to assess the treatment outcome 14 days after embryos transfer. A diagnosis of pregnancy was confirmed by a rise in β-HCG (>20 IU) in 2 consecutive blood tests or a positive urinary outcome. Pregnancies were considered as visualization by gestational sac on ultrasound and fetal heart movements at ≥6 weeks' gestation.

2.5. Statistical analysis

All data analysis was performed using SPSS. 11.0 software (SPSS, Chicago, IL). Continuous variables were expressed as means ± standard deviation and analyzed using the independent Student *t* test. The Spearman rank correlation coefficient was calculated to assess the association between lipid level and embryo quality. A value of $P < .05$ was considered to be statistically significant.

3. Results

3.1. Baseline characteristics

A total of 488 patients were included in this study. Patients were divided into 2 groups: pregnant (n = 286) and nonpregnant (n = 202) groups according to the pregnant outcomes. The general characteristics of patients were presented in Table 1. There were no statistical differences between the 2 groups with regard to the age, body mass index (BMI), number of antral follicles, and sex hormones including FSH, luteinizing hormone, and estradiol ($P > .05$). No differences were observed in term of the lipid levels such as TG, TC, HDL, LDL, Lp(a) and Lp(b) ($P > .05$) between the 2 groups.

3.2. Comparison of embryo outcomes between the 2 groups

The embryo outcome in pregnant and nonpregnant groups were shown in Table 2. No significant differences in the number of oocytes, number of normal fertilized oocytes and number of cleavage embryos were observed between 2 groups. However, the normal fertility rate in pregnant group (0.80% ± 0.16%) was significantly higher than nonpregnant group (0.78% ± 0.14%) ($P < .05$). For the quality of embryos, there were no significant differences in the number of the cleavage embryos and number of good quality embryos between 2 groups. The pregnant group had a significantly higher good quality embryo rate than that in nonpregnant group (0.68% ± 0.23% vs 0.59% ± 0.26%) ($P < .05$).

Table 1

The general characteristics of patients (means ± SD).

	Pregnant group	Nonpregnant group	*P*-value
Cycle characteristics			
Cycle (n)	286	202	
Age, yr	30.48 ± 4.05	30.00 ± 3.85	.499
Body mass index, kg/m^2	22.23 ± 3.16	22.54 ± 3.30	.483
Basal FSH, mIU/mL	6.57 ± 1.58	6.68 ± 1.32	.860
LH, mIU/mL	2.90 ± 1.58	2.86 ± 1.41	.999
FSH/LH	2.90 ± 1.50	2.80 ± 1.16	.402
No. of antral follicles	14.77 ± 4.90	14.50 ± 5.00	.464
E_2, pg/mL	42.86 ± 15.17	44.91 ± 15.56	.308
Lipid levels			
TC, mMol/L	4.29 ± 0.85	4.42 ± 0.75	.349
HDL, mMol/L	1.37 ± 0.32	1.42 ± 0.36	.242
TG, mMol/L	1.27 ± 0.64	1.01 ± 0.59	.081
LDL, mMol/L	2.18 ± 0.62	2.25 ± 0.56	.629
Lp(a), mMol/L	1.16 ± 0.19	1.20 ± 0.24	.061
Lp(b), mMol/L	0.78 ± 0.18	0.78 ± 0.16	.957

FSH = follicle-stimulating hormone, HDL = high-density lipoproteins, LDL = low-density lipoprotein, LH = luteinizing hormone, SD = standard deviation, TC = total cholesterol, TG = triglycerides.

Table 2

The comparison of embryo outcome between pregnant and nonpregnant groups (means ± SD).

	Pregnant group	Nonpregnant group	*P*-value
Cycle (n)	286	202	
No. of oocytes	10.82 ± 3.90	11.46 ± 4.12	.382
No. of normal fertilized oocytes	8.58 ± 3.38	8.85 ± 3.52	.841
Normal fertility rate (%)	0.80 ± 0.16	0.78 ± 0.14	.028
No. of cleavage embryos	8.37 ± 3.33	8.63 ± 3.41	.860
Cleavage rate (%)	0.98 ± 0.06	0.98 ± 0.05	.882
No. of good quality embryos	5.63 ± 2.95	4.99 ± 3.04	.062
Good quality embryos rate (%)	0.68 ± 0.23	0.59 ± 0.26	.002

SD = standard deviation.

3.3. Correlation between lipid level and embryo quality

Spearman correlation analysis between the lipid level and embryo quality in pregnant group was analyzed in this study. As shown in Table 3, BMI correlated negatively with number of normal fertilized oocytes, number of cleavage embryos and number of good quality embryos ($P < .05$). The levels of serum TG, TC, and LDL were negatively correlated with number of normal fertilized oocytes ($P < .05$). TG, TC, and Lp(b) were negatively correlated with number of good quality embryos ($P < .05$). Additionally, TG was negatively correlated with number of oocytes and number of cleavage embryos ($P < .05$). However, HDL and Lp(a) had positive correlation with the number of oocytes, the number of normal fertilized oocytes, and the number of cleavage embryos ($P < .05$).

4. Discussion

Dyslipidemia may influence the oocyte quality and fertility, and accumulating studies indicate that dyslipidemia plays a potential role in the failure to fertility through inducing oxidative stress.[15] Therefore, we believed that the quality of embryo might be associated with lipid abnormalities in infertility patients. However, the association between lipid parameters and embryo quality was rarely investigated. In the present study, we found that serum levels of TG, TC, LDL, and Lp(b) were negatively correlated with the embryo quality, while the serum levels of HDL and Lp(a) were positively correlated with the embryo quality.

HDL played a central role in the process of dyslipidemia in infertility patients.[16] HDL particles consist of a phospholipid bilayer and core, protein components such as apolipoproteins (Apo AI and AII), as well as lipolytic and antioxidant enzymes.[17] The role of HDL in the process of mammalian female reproduction has been demonstrated in knockout (SR-BI KO) female mice. Animal experiments indicated a high incidence of exencephaly with female bias in embryos lacking SR-BI, which is related to the aberrant HDL metabolism.[13] High incidence of extracerebral malformations showed gender bias in women. Moreover, follicular fluid HDL cholesterol, apolipoprotein A-1 as well as follicular fluid β – cryptoxanthin and gtocopherol have been regarded as predictors of embryo quality (mainly about embryo fragmentation) after IVF. Browne et al confirmed that HDL exhibit important cytoprotective effect on oocytes and surrounding granulosa cells.[18] These findings prompted us to further study the association between the HDL and embryo development. In the present study, we observed that the level of serum HDL was positively correlated with number of oocytes, number of normal fertilized oocytes and cleavage embryo numbers. This result indicated that HDL might be protective in development from oocyte to embryo. This protective effect may be associated with the delivery of cholesterol to corpus luteum as a substrate for the synthesis of progesterone by both HDL and LDL.[12] However, this observation remain to be further demonstrated.

Table 3

Correlation between lipid level and embryo quality in pregnant group.

		BMI	TG	TC	HDL	LDL	Lp(a)	Lp(b)
No. of oocytes	Correlation Coefficient	+0.030	−0.081	−0.024	+0.047	+0.006	+0.058	+0.037
	P-value	.582	.007	.613	.032	.835	.005	.792
No. of normal fertilized oocytes	Correlation Coefficient	−0.041	−0.123	−0.050	+0.113	−0.040	+0.076	−0.021
	P-value	.033	.005	.035	.006	.041	.008	.709
Fertility rate	Correlation Coefficient	−0.057	−0.049	+0.010	+0.067	−0.006	+0.016	−0.055
	P-value	.721	.683	.842	.770	.955	.811	.767
No. of cleavage embryos	Correlation Coefficient	−0.056	−0.128	−0.039	+0.137	−0.031	+0.100	−0.017
	P-value	.005	.003	.704	.003	.698	.004	.675
No. of good quality embryo	Correlation Coefficient	−0.086	−0.135	−0.078	+0.053	−0.046	−0.034	−0.105
	P-value	.004	.002	.003	.643	.728	.875	.002
Good quality embryo rate	Correlation Coefficient	+0.038	−0.117	−0.076	+0.018	−0.044	−0.045	−0.068
	P-value	.692	.853	.712	.877	.726	.689	.933

+/− indicate positive or negative correlationship.

BMI = body mass index, HDL = high-density lipoproteins, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglycerides.

TC and TG were 2 important components of dyslipidemia. The negative associations between TG levels with number of oocytes, cleavage embryos, normal fertilized oocytes, and good quality embryos of women in our study were consistent with a previous result.[19] In addition, TC levels were found to be negatively correlated with normal fertilized oocytes numbers and good quality embryos numbers. Some observations regarding the development of human follicle suggest governing in vitro human embryo fragmentation may depend on the cholesterol metabolism, while the processes that regulating embryo cell division rate may be independent of the cholesterol metabolism.[20]

There has been increasing evidence regarding the negative effects of raised BMI on IVF outcomes.[21,22] Our analysis results, in line with the previous findings, indicated that BMI was negatively associated with the number of normal fertilized oocytes, cleavage embryos, and good quality embryos. Endometriosis has been well known to be a common risk factor for infertility. It was demonstrated recently that high BMI might seriously influence the pregnancy rate of IVF in patients with endometriosis.[23] Additionally, it was observed in couples undergoing intracytoplasmic sperm injection that raised paternal BMI has a negative impact on clinical pregnancy rate and live birth rate.[24]

Previously, it was found that physiological changes in lipid profile were involved in lipoprotein metabolism and might lead to adverse pregnancy outcomes.[25] The relationship between lipid level and embryo quality was compared and analyzed in our present study. The results suggested that Lp(a) was positively related to number of oocytes, normal fertilized oocytes, and cleavage embryos, while Lp(b) was only negatively related to the of good quality embryo numbers. Interestingly, neither Lp(a) nor Lp(b) was associated with the fertility rate or the good quality embryo rate. Previously, Todoric et al reported that Lp(a) level had no significant difference in pregnant women with and without gestational diabetes.[26,27] This might be explained by the fact that Lp(a) level did not exceed the threshold of 46 mg/dL although it was higher than the physiological concentrations.

One of the limitation was that we did not find a significant relationship between LDL and embryo morphology parameters, except for the number of normal fertilized oocytes. Our findings indicated that LDL should not be a key potential factor for the infertility related to the dyslipidemia. Another limitation was sources of potential bias or imprecision.

Overall, the levels of serum TG, TC, LDL, and Lp(b) were negatively correlated with the embryo quality, and the levels of serum HDL and Lp(a) were positively correlated with the embryo quality. Our present findings may shed an insight into the potential effect lipid profile during pregnancy on adverse pregnancy outcomes.

Author contributions

SW and JW conceived the study. SW performed the literature search and writing of the manuscript. SW and JW analyzed and interpreted the data. YJ and WJ collected and assembled the data. SW submitted the manuscript and is the corresponding author. All authors read and approved the final manuscript.

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