Evaluation of Lipids in Serum and Follicular Fluid on Oocyte and Human Embryo Quality after ICSI

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Background: The estimation of oocyte quality in human *in vitro* fertilization (IVF) is an important point for the embryologists. Oocyte selection and the identification of the best oocytes may help to limit an overproduction of embryos and to improve the results of oocyte cryopreservation programs.

The follicular fluid (FF) can be provided easily during oocyte pick-up and known to represents an optimal source for non-invasive biochemical predictors of oocyte quality. However, till now no substance was found to be used as reliable markers of oocyte competence to fertilization, embryo development and pregnancy.

Metabolism and ATP levels within the oocyte and adjacent cumulus cells are associated with quality of oocyte and optimal development of a healthy embryo. Lipid metabolism provides a potent source of energy and its importance during oocyte maturation is being increasingly recognised.

Objectives: To determine the effect of lipids (Cholesterol. Triglyceride and HDL) level in serum and follicular fluid in predicting oocyte quality, embryo quality and outcome in patients undergoing intracytoplasmic sperm injection program

Patients and Methods: Fifty eight (58) infertile women with an age range 21-41 years old undergoing intracytoplasmic sperm injection cycles were included in this study. Lipids (Cholesterol. TG and HDL.,) levels in serum and follicular fluid were measured by auto analyzer for biochemistry (Flexor El_200) and correlation with oocyte quality, embryo quality and ICSI outcome was done.

Results: The results of this study showed no significant ($p<0.05$) difference in lipid levels in serum and follicular fluid between pregnant and non pregnant females as well as non significant correlation was found between lipid level in serum and follicular fluid in relation to oocyte quality and embryo quality.

Conclusions: Serum and follicular fluid lipids are not good predictors of oocyte quality, embryo quality or ICSI outcome in women undergoing IVF/ICSI cycles. Accordingly, these biomarkers would be less reflective of the follicular environment and that the effect of maternal metabolic disorders on oocyte quality and fertility outcome is complex and of a multifactorial kind.

Keywords: **Lipids, follicular fluid, oocyte quality, embryo quality.**

Introduction:

Infertility indicates a difficulty to conceive or carrying a pregnancy to term, but it is not synonymous with sterility (the inability to reproduce [1]. Assisted Reproductive Techniques (ART) are methods used to achieve pregnancy by artificial or partially artificial measures. During ICSI, the oocytes are collected by transvaginal ultrasound guided aspiration of the follicular fluid (FF). The oocytes are transferred to suitable culture medium and are inseminated by intracytoplasmic sperm injection (ICSI), whereby single spermatozoa are injected directly into mature oocytes [2]). FF is a suitable environment for the growth and development of oocytes. It is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretary activity of granulosa and theca cells [3].

Oocytes and embryos are suggested to be highly sensitive to any change in their environment caused by metabolic, dietary or other factors, thereby having fatal consequences for the final fertility [4]. Lipid composition of follicular fluid, to which the preovulatory follicle is exposed, is one of the major factors determining subsequent fertility. Lipids are not only fuels and membrane constituents, but also precursors of signalling molecules involved in dominance, ovulation and atresia mechanisms [5]).

Analyses of lipoproteins in human follicular fluid showed that HDLs but little or no LDL or VLDL are present[6]. Follicular fluid HDL cholesterol is positively correlated with serum HDL cholesterol[7], indicating that HDL particles are serum derived and passively equilibrated; however, there is no similar correlation for VLDL cholesterol in follicular fluid and serum[8]. Thus, it is generally accepted that in mammals HDL is the sole lipoprotein present in follicular fluid related to the porosity of the follicle basement membrane which is permeable to serum proteins up to 300 kDa in size[6], thus LDL and VLDL are excluded. Although some studies detected LDL and/or VLDL in follicular fluid of some women[9] and it is also reported that human granulosa-lutein cells express lipoprotein marker ApoB-100 and assemble and secrete *in vitro*-native VLDL particles similar to those in serum, except with slightly higher triglyceride content and less cholesterol[8]. Thus, LDL and VLDL particles detected in follicular fluid may in fact be generated by ovarian cells [6].

Patients and Methods:

This study included fifty eight women who underwent ICSI at the infertility clinic of High Institute of Infertility Treatment and Assisted reproductive technologies/ Al- Nahrain University/ Iraq, and Kamal Al-Samarrai hospital for infertility treatment/ Iraq. Their mean age was 33.56± 0.69 years (mean±SEM) (range: 21 to 41 years). Eighteen women (31%) achieved pregnancy and was defined as pregnant group (group 1), while forty women (69%) failed in achieving pregnancy who defined as non-pregnant group (group 2).

Intracytoplasmic Sperm Injection Procedure:

Controlled Ovarian Stimulation (COS):

All patients included in this study were subjected to long agonist protocol. After

selection, women received mid- luteal protocol down-regulation with GnRH agonist triptorelin (Decapeptyl 0.1 mg, Ferring Co, Kiel, Germany) by daily subcutaneous injection and the pituitary desensitization was completed by reaching the level of E2 < 50 pg/ml and endometrial thickness was ≤ 4 mm on ultrasound examination, the women received rFSH (Gonal F, Merck Serono) containing 75 IU of FSH activity per ampoule by daily subcutaneous injection (2-5) ampoules according to age and weight . Transvaginal ultrasound (TVU) was performed on cycle day 7 and subsequent scan were done every 2-3 days as required. The doses of (Gonal F) and follicle growth were monitored by serial serum E2 level and TVU till the day of hCG administration. Then, ovulation was induced by administration of rhCG, (Ovitrelle 6500 IU; Merck Serono) subcutaneously when at least (3-4) follicles > 17 mm in diameter were detected on ultrasound examination.

Aspiration of the oocytes and Intracytoplasmic Sperm Injection:

Oocyte retrieval was performed by single lumen aspiration needle under vaginal ultrasound-guide 34-36 hours after hCG injection under general anaesthesia. All patients received progesterone daily starting from the day of the ova pick up (OPU). Under the inverted microscope at 100×, the oocytes are scored as immature (prophase I), intermediate (metaphase I), mature (metaphase II), and atretic. Thereafter, the oocytes are incubated in CO_2 incubator for (30 minutes -1 hours). Immediately prior to micromanipulation, each oocyte is examined by the embryologist in IVF laboratory under the microscope to assess the maturation stage, which is either GV or germinal vesicle or MI or metaphase I in which there is no germinal vesicle or polar body or metaphase II (MII) being assessed according to the absence of the germinal vesicle and the presence of one polar body. After sperm injection the embryo quality is checked in the next 1-3 days under the inverted microscope (ICSI microscope). Luteal phase supported by oral progestogens.

Collection of Serum and Follicular Fluid:

Blood samples of 58 women were obtained on day of OPU and left in plain tube. The blood was allowed to coagulate for 30 minutes and then centrifuged to separate the serum for 15 minutes at 3000 rpm. Follicular fluid was obtained from the first retrieved follicle to avoid contamination of blood and media used during aspiration and collected in special conical tubes.

Lipids estimation in Serum and Follicular Fluid:

Serum and FF lipids concentrations were determined by autoanalyser for biochemistry (Flexor El_200).

Statistical analysis:

Data were summarized, presented and analyzed using statistical package for social science (spss) version 23 and Microsoft office Excel 2017. Numeric variables were expressed as mean± standard error (SE), while nominal variables were expressed as number and percentage. Independent sample student t-test was used to compare mean between any two groups.

Results:

Comparison of serum & follicular fluid lipids' levels between pregnant and non-pregnant groups:

Results are expressed as means ± SEM. The overall mean concentration ± SEM of each type of lipid was calculated for follicular fluid and for blood serum in all females.

- Serum lipids levels (table -1):

1-Serum Cholesterol level in all patients shared in this study was (148±5.3) mg/dl. For group (1), it was (142.5 ±8.19) mg/dl, while in group (2) was (151.8 ±6.7) mg/dl.

2- Serum triglyceride (T.G) level in all patients shared in this study was (119.8 ±5.9) mg/dl. For group (1), it was (121 ±12.4) mg/dl, while in group (2) was (119.1 ±6.6) mg/dl.

3- Serum HDL level in all patients shared in this study was (45.8 ±1.5) mg/dl. For group (1), it was (47.2 ±2.4) mg/dl, while in group (2) was (45.2 ±1.9) mg/dl.

The statistical analysis for all parameters showed non significant (P>0.05) difference between the pregnant and non-pregnant groups (figure 1).

Table (1): Comparison of mean Serum lipids levels between pregnant and non-pregnant groups:

Characteristic	Non Pregnant (n=40) Mean± SE	Pregnant (n = 18) Mean± SE	Total Mean± SE
Serum Cholesterol (mg/ml)	151.8± 6.7	142.5± 8.19	148.9± 5.3
Serum Triglyceride (mg/ml)	119.1± 6.6	121.3± 12.4	119.8± 5.9
Serum HDL (mg/ml)	45.2± 1.9	47.2± 2.4	45.8± 1.5

- Follicular Fluid lipids level:

Table (2) shows the **(**mean± SE) of different lipid levels in follicular fluid

1- Cholesterol level in all patients included in this study was (19.9 ±2.7) mg/dl. For group (1) it was (24.7 ±8.4) mg/dl, while in group (2) it was (17.7 ±1.2) mg/dl.

2- T.G level in all patients included in this study was (9.1 ±0.6) mg/dl. For group (1), it was (8.3 ±0.9) mg/dl, while in group (2) was (9.4 ±0.7) mg/dl.

3- HDL level in all patients present in this study was (9.8 ±0.5) mg/dl. For group (1), it was (8.8 ±0.8) mg/dl, while in group (2) was (10.3±0.6) mg/dl.

The statistical analysis showed non significant (P> 0.05) difference between the pregnant and non-pregnant groups for all the previously mentioned parameters (figure-2).

Table (2): Comparison of mean follicular fluid lipids levels between pregnant and non-pregnant groups:

Characteristic	Non Pregnant (n=40) Mean± SE	Pregnant (n = 18) Mean± SE	Total Mean± SE
FF Cholesterol (mg/ml)	17.7± 1.2	24.7± 8.4	19.9± 2.7
FF Triglyceride (mg/ml)	9.4± 0.7	8.3± 0.9	9.1± 0.6
FF HDL (mg/ml)	10.3± 0.6	8.8± 0.8	9.84± 0.5

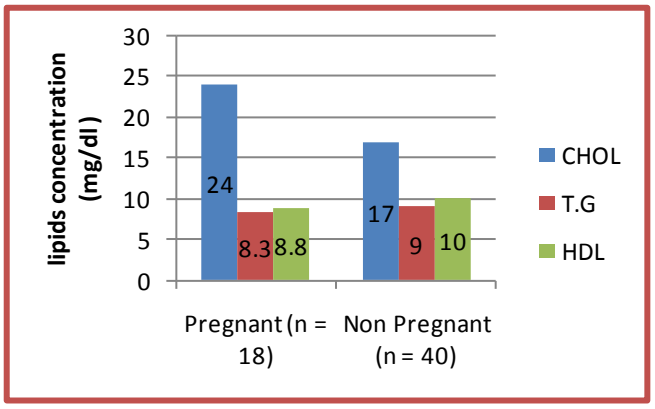

Figure (1) Comparison of mean serum lipids levels between pregnant and non-pregnant groups.

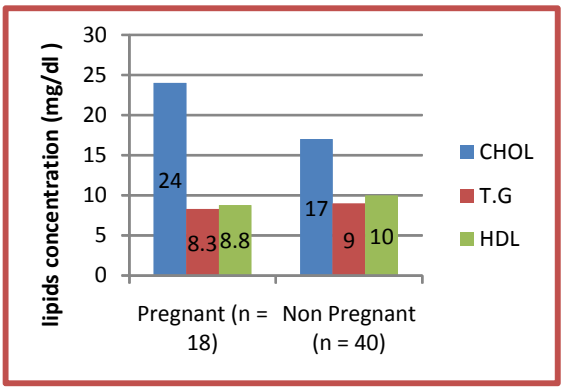

Figure (2) Comparison of mean follicular fluid lipids levels between pregnant and non-pregnant groups.

Correlation of serum lipids levels at the day of OPU and other clinical and cycle parameters in all infertile females as shown in Table(3) :

1. There is a non significant correlation between the FF. Cholesterol level and the **MII oocyte**. (r= -0.045, P = 0.736).
2. There is a non significant correlation between the FF. Cholesterol level and **Fertilization Rate** . (r=-0.202, P=0.128).
3. There is non significant correlation between the FF. Cholesterol level and **Grade I embryo**. (r=-0.191, P =0.380).
4. There was non significant correlation between the FF. T.G . level and the **MII oocyte** (r= -0.051, P = 0.701).
5. There was non significant correlation between the FF. T.G. level and **Fertilization Rate**. (r=-0.113, P=0.399).
6. There is non significant correlation between the FF. T.G. level and **Grade I embryo** . (r=-0.026, P =0.957).
7. There is a non significant correlation between the FF. HDL. level and the **MII oocyte**. (r= -0.095, P = 0.478).
8. There is a non significant correlation between the FF. HDL. level and **Fertilization Rate**. (r=-0.001, P=0.994).
9. There is non significant correlation between the FF. HDL. level and **Grade I embryo**. (r=-0.119, P =0.154).

Table (3): Correlation of different lipids levels in follicular fluid at the day of OPU and other clinical and cycle parameters in all infertile females.

Correlation		MII oocyte	Grade I Embryo	Fertilization Rate
FF. Triglyceride	(r)	-0.051	- 0.026	0.113
	P-value	0.701	0.957	0.399
FF. Cholesterol	(r)	0.045	- 0.191	0.202
	P-value	0.736	0.380	0.128
FF. HDL	(r)	0.095	0.119	0.001
	P-value	0.478	0.154	0.994

(r) Pearson Correlation

Discussion:

Follicular fluid is in part exudates of serum and is also partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells [10]. It is well known that both the oocyte and the embryo are very susceptible to changes in their micro-environment [11]. +Recent research has been concentrating on the follicular micro-environment of women undergoing assisted reproductive treatment [12]. An alteration in the composition of this microenvironment might affect on oocyte and cumulus cell quality [13]

Comparison of serum and follicular fluid lipids between pregnant and non pregnant groups:

The results of this study showed no significant difference in mean serum and follicular fluid cholesterol level between the pregnant and non-pregnant groups. These results agreed with previous studies done by Saime and Meltem who documented the absence of any differences in serum cholesterol level on fertility, suggesting that the lipids taken into consideration seem to be of no significance in the ability of oocyte to be successfully fertilized *in vitro* [14].

The cholesterol level in follicular fluid of all females included in this study showed no significant difference between pregnant and non pregnant groups, this finding was in agreement with previous study by Nandi *et al* who stated that there was no relation between follicular fluid content of cholesterol and fertility[15]. However, the result of the present study differed from that reported in Thangavel & Nayeem who reported decrease cholesterol level in follicular fluid of mature oocyte is supposed to have more fertilization competence. The decreased cholesterol level in the large follicles in those studies might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis [16].

Recent analyses have demonstrated that in human follicular fluid, most serum metabolites including triacylglycerol and free fatty acids are reflected in follicular fluid, but at lower levels and exhibit weak but significant correlations with levels in serum[12] confirming a modulating role for the blood-follicular fluid barrier and/or a substantial contribution of oocyte, granulosa and cumulus cell metabolism [17].

In the present study it was also found that there was no significant difference in triglyceride level both in serum and follicular fluid between pregnant and non pregnant groups (table-1). These findings are similar to that of Valckx *et al* 2012 who suggested serum and follicular fluid TG was not associated with any reproductive outcome [7].

HDL cholesterol was measured in serum and follicular fluid of all females included in this study. The results showed no significant difference between pregnant and non pregnant groups (table 1). These finding were in coordinate with that of previous studies which stated that in several populations of familial HDL deficiency, there is evidence for fertility in women suffering from reduced plasma HDL cholesterol levels. The latter suggests that HDL cholesterol is not obligatory for reproductive potential in the human female, but may play a role in reproductive aging and oocyte health [18]. However other studies provided further support that plasma substrates such as HDL particles transported to developing ovarian follicles from plasma may be important for oocyte development as phospholipid associated with FF HDL, has been shown to induce endothelial proliferation and angiogenesis in the vasculature surrounding the follicle [18] in addition to demonstrating anti-apoptotic and antioxidant properties [19].

However, two recent studies have reported that increased HDL and Apo. AI levels were associated with failure of oocytes to cleave and decreased the numbers of good quality embryos. In addition, it is unclear whether lipoproteins found in follicular fluid influence oocyte quality via their ability to deliver lipid

substrates such as triacylglycerol or whether other components of these particles, namely the surrounding apolipoproteins, which are known to have scavenging properties, protect cells from oxidative stress [20, 21].

Correlation Serum and Follicular Fluid parameters (lipid) levels with Oocyte quality and Embryo Quality:

The crucial test (or the gold standard) for oocyte quality is its ability to be fertilized, to develop to the blastocyst stage and finally to create a pregnancy resulting in living offspring [22]. Similarly, several invasive and non-invasive parameters (morphology, cell number, developmental kinetics, apoptosis, genetic anomalies …) have been described to evaluate embryo quality [23]. However, embryo quality is mostly determined by the culture environment and less by the oocyte's origin [22, 24]. Thus, the post-fertilization micro-environment in the oviduct and uterus is crucial and has a major impact on embryo quality (metabolism and gene expression) [25].

In table (2) the present study there was no significant correlation between the lipids (cholesterol, triglyceride and HDL) both in serum and follicular fluid with MII oocyte and grade I embryo and also on fertilization rate. These findings were in agreement with previous studies which recorded non significant correlation between any follicular fluid lipid and fertilization competence of oocytes and embryo cleavage rate number of transferred embryos [26].

These results was not in agreement with that of most previous studies which documented that lipoproteins derived from blood that are established in follicular fluid and may be responsible for o oocyte development. Whether lipoproteins transport triacylglycerides to ovarian cells for energy production similar to their roles in other cells is less clear, but there is rising evidence for this as an important metabolic pathway in maturing ovarian follicles. Within cells, triacylglycerides are stored in lipid droplets and these are prevalent and being characterised molecularly in the oocytes and cumulus cells of many species. Much remains to be determined about how the metabolism of triacylglycerides by lipolysis and fatty acids by b-oxidation is regulated in cumulus cells and oocytes and the relative importance of this form of energy production for female fertility [6].

Thus, in the present study the estimates of biologic variability were vague, and it was difficult to characterize FF HDL analytes by relevant demographic and clinical factors, such as infertility diagnosis (e.g., diminished ovarian reserve) and COS protocol relevant to IVF. A larger sample size will be needed to more definitively investigate the clinical relevance of these results for IVF. As well as it is reported that levels of HDL components measured in mammalian FF depend on follicle size[15], yet diameter data were unavailable for incorporation into the analysis.

The present study has several limitations, and thus, the results should be interpreted with caution. Most importantly, was the very limited sample size as follicular fluid samples were collected from only fifty eight infertile females.

Conclusions: Serum and follicular fluid lipids are not good predictors of oocyte quality, embryo quality or ICSI outcome in women undergoing IVF/ICSI cycles. Accordingly, these biomarkers would be less reflective of the follicular environment and that the effect of

maternal metabolic disorders on oocyte quality and fertility outcome is complex and of a multifactorial kind.

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