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The Journal of Obstetrics and Gynaecology

Journal of Obstetrics and Gynaecology

ISSN: 0144-3615 (Print) 1364-6893 (Online) Journal homepage: http://www.tandfonline.com/loi/ijog20

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To cite this article: S. Fayezi, M. Darabi, M. Darabi, M. Nouri, A. Rahimipour & A. Mehdizadeh (2014) Analysis of follicular fluid total phospholipids in women undergoing in-vitro fertilisation, Journal of Obstetrics and Gynaecology, 34:3, 259-262, DOI: 10.3109/01443615.2013.851657

To link to this article: http://dx.doi.org/10.3109/01443615.2013.851657

Published online: 29 Jan 2014.

Journal of Obstetrics and Gynaecology, 2014; 34: 259–262

ISSN 0144-3615 print/ISSN 1364-6893 online
DOI: 10.3109/01443615.2013.851657

informa
healthcare

GYNAECOLOGY

Analysis of follicular fluid total phospholipids in women undergoing *in-vitro* fertilisation

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Follicular fluid (FF) samples were obtained from 100 patients referred to the University Hospital. A total of 79 subjects underwent IVF and the remaining 21 underwent ICSI. The levels of apoA-I and total phospholipid were measured using turbidometric and colorimetric phosphorus assays, respectively. Correlation analysis showed a significant inverse association of total phospholipid in FF with fertilisation ratio ($r = -0.24$, $p = 0.04$). Furthermore, the ratio of phospholipid/apoA-I in patients with a percentage of fertilised oocytes ≤ 50% was significantly higher (> 2.5%, $p < 0.05$) than in those with higher percentages of fertilised oocytes. The amounts of phospholipid and phospholipid/apoA-I ratio in FF were associated negatively to the percentage of oocyte fertilisation. Therefore, the change in the phospholipid and phospholipid/apoA-I ratio of FF might be regarded as indicators of female fertility.

Keywords: ART, follicular fluid, phospholipid

Introduction

In recent decades, extrauterine techniques such as *in-vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) have been used to treat infertility. The underlying success rate of these techniques is considered via laboratory and clinical success (Kallen 2008). Zygote cell formation and pregnancy occurrence account for laboratory and clinical success, respectively (Kallen 2008; Son et al. 2013). Biological membranes are fluid lipid bilayers, which maintain the integrity of the cell or organelles and act as barriers against polar molecules and ions. Phospholipids are a major structural component of cellular membrane and organelle membranes and are involved in the modulation of multiple cellular interactions, such as cell–cell and cell–matrix interactions (Mannock et al. 2010).

Follicular fluid (FF) is a liquid which plays a key role in the nutritional and developmental support of the oocyte (Wallace et al. 2012). Follicular fluid supplementation of *in-vitro* maturation medium has been implicated in the improvement of development of oocytes and fertilisation (Somfai et al. 2012). Follicular fluid contains a significant amount of phospholipid compounds, which may affect cellular interactions and fusion events (Tokumura et al. 1999). For example, platelet activating factor (PAF), a naturally occurring phospholipid in FF, has a significant positive effect on ovulation and is considered as the key sperm capacitation factor in IVF (Ali et al. 2007; Xu et al. 2009). Accordingly, Buckingham and colleagues (2006) have shown that antiphospholipid antibodies in human FF are associated with decreased implantation and fertilisation rates. Phospholipids are precursors of the biologically active compounds, eicosanoids, which contribute to fertility (Sato et al. 2011). It therefore seems that the level of phospholipid in FF can affect the zygote maturity process and thus the fertilisation rate.

Apolipoprotein A-I (apoA-I) is the major apolipoprotein in FF, which is associated with high density lipoprotein (HDL) (Fujimoto et al. 2010). ApoA-I is synthesised by cumulus cells and could support the growth of oocytes by facilitating transfer and redistribution of lipids (Choi et al. 2010). ApoA-I in FF plays a key role in transferring cholesterol to granulosa cells, providing precursors for the synthesis of steroid hormones that regulate female fertility. Recently, it has been shown that cholesterol transport activity of FF has a direct correlation with the fertility rate (Mehdizadeh et al. 2011). Age-dependent decreases in the apoA-I and increases in the pro-inflammatory factors in the FF have also been associated with lower oocyte maturity and fertility rates (Von et al. 2010).

It has been shown that phospholipid fraction of HDL particles plays an important role in lipoprotein cholesterol transport through interaction with apoA-I (Rye et al. 2002). Since FF phospholipid is almost exclusively associated with apoA-I in HDL particle structure, it seems that the interaction effect of phospholipid and apoA-I may affect fertility. The aim of this study was to investigate the correlation between phospholipid levels in FF and the success rate of IVF/ICSI techniques in patients undergoing these techniques.

Materials and methods

Patients

The subjects consisted of 100 patients referred to the University Hospital in 2007–2009 and were the same as those investigated in a previous study (Shaaker et al. 2012). The study was approved by the ethics committee of Tabriz University of Medical Sciences. Before sampling, written consent was taken from each participant. All participants underwent a screening examination, including medical history, physical examination, measurements of serum hormone levels and blood counts. A total of 79 patients underwent IVF and the remaining 21 underwent ICSI. The mean

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age of the subjects was 31.7 ± 5.46 years, with no evidence of any disease. Non-smoking, healthy husbands were defined as including criteria. Uterine abnormalities, positive history of endocrine disease and inflammatory disorders such as thyroid and adrenal disorders, immune system defect and sexual hormones disorders were considered as exclusion criteria in this study.

Oocyte retrieval

Ovarian stimulation was achieved with a GnRH agonist (Decapeptyl; Debio Pharm, Geneva, Switzerland)/FSH-long downregulation protocol (Shaaker et al. 2012). Controlled ovarian stimulation was started with recombinant human follicle stimulating hormone (rFSH, Gonal-F; Serono, Switzerland) on the 3rd day of the menstrual cycle. The daily rFSH dose ranged between 150 and 300 IU, depending on body mass index, age of the women and the anticipated ovarian response. Dose adjustment was done according to follicular development and serum oestradiol levels. Intramuscular hCG (1,000 IU, Choriomon, Meizler, Brazil) was administered when sonography revealed three preovulatory follicles with an average diameter of 18–20 mm. Oocyte retrieval was done 36 h after hCG administration by vaginal ultrasound-guided puncture of the ovarian follicles. The collected oocytes were incubated in 37°C with 5% CO_2 for 4 h and were then used for IVF and ICSI. Follicular fluid sample were collected in a sterile tube, centrifuged at 500*g* for 5 min and kept frozen at −70°C until analyses.

Oocytes with sporadic cumulus oophorus and zona pellucida and also a clear ooplasm were selected for insemination. The swim-up was used to prepare sperm for IVF (Jakab et al. 2003). Retrieved oocytes were inseminated with 250,000 sperm per oocyte in the IVF technique. In the ICSI group, a single motile sperm was injected into oocytes. After 24 h, the oocytes were separated from surrounding granulosa cells. A maximum of three embryos were transferred at 4–8 cells' stages after 48 h, under ultrasound guidance. Clinical pregnancy was assessed by β-hCG test, 14 days after embryo transfer. The fertilisation rate was defined as the proportion of oocytes with two pronuclei.

Laboratory measurements

ApoA-I concentration in FF samples were measured by immunoturbidimetric methods (DiaSys Diagnostics, Holzheim, DE, USA) on a Abbott Alcyon 300 Analyser (Abbott Laboratories, Abbott Park, IL, USA). Total lipids from the FF supernatant were extracted by the method of Bligh/Dyer with a 2:1 chloroform:methanol solution and several washes (Bligh and Dyer 1959). FF total phospholipid was separated by one-dimensional thin-layer chromatography by using silica gel 60 G plates (E. Merck, Darmstadt, DE, USA) and an 80:20:1 hexane:ethyl ether:acetic acid development solvent. Phosphorus content of phospholipid was estimated after hydrolysis with concentrated perchloric acid at 180°C for 2 h (Chen et al. 1956). The overall coefficients of variation were 6.5% for phospholipid and 5.9% for apoA-I.

Data analyses

Values are presented as mean ± SD. The one-way ANOVA with Tukey *post hoc* pair-wise comparison and multivariate analysis of variance and *t*-test were used for comparing means and ratios in different groups. Statistical relationship between fertility indexes and variables were analysed by Correlation test. A p value of < 0.05 was considered statistically significant. Analysis was carried out using SPSS 11.5 statistical software.

Results

General characteristics, husbands' spermogram and FF parameters are shown in Table I. The average numbers of mature oocytes and of fertilised oocytes were about 8.5 and 5.0, respectively. The pregnancy rate in the studied population was 24.0%.

Table I. Patients' clinical characteristics, biochemical profile of follicular fluid and semen parameters of husbands.

	Mean ± SD	Range
Age (years)	31.7 ± 5.46	21–30
Body mass index (kg/m^2)	25.5 ± 3.13	20–34
IVF parameters		
Mature oocytes	8.57 ± 4.19	1–19
Fertilised oocytes	5.0 ± 3.0	0–13
Pregnancy (%)	24	
Biochemical profile of follicular fluid		
HDL-C	26 ± 10	9–24
Apolipoprotein A-I (mg/dl)	105 ± 19	14–133
Total phospholipid (mg/dl)	33.61 ± 6.54	17.5–42.3
Total phospholipid/apolipoprotein A-I	0.34 ± 0.14	0.1–1.2
Semen characteristics	0.33 ± 0.13	0.14–1.17
Sperm count (10^6/ml)	59.5 ± 30.3	28–120
Sperm mobility (%)	66.1 ± 18.4	10–90

As shown in Table II, the age of patients was inversely associated with the number of mature oocytes ($r = -0.21$, $p = 0.04$). However, there was no significant correlation of the number of mature oocytes with the levels of apoA-I or total phospholipid.

The fertility rate (FR) was calculated as the percentage of fertilised oocytes at the pronuclear stage after IVF. As shown in Table III, BMI ($r = -0.27$, $p = 0.02$), total phospholipid ($r = -0.24$, $p = 0.04$) and the ratio of phospholipid/apoA-I ($r = -0.24$, $p = 0.04$) were inversely correlated with FR in the IVF group. Sperm motility percentage showed a trend to significant positive correlation ($r = 0.27$, $p = 0.05$) with FR.

To further investigate the relation of phospholipid/apoA-I with FR and whether there might be a threshold effect, patients were classified into three groups according to the index of fertility (IFR) based on the FR (IFR-I, < 50%; IFR-II, 51–69%; IFR-III, > 70%). After matching BMI, sperm count and sperm motility, the ratio of phospholipid/apoA-I in patients with a percentage of fertilised oocytes ≤ 50% was significantly higher (> 2.5%, $p < 0.05$) than in those with higher percentages of fertilised oocytes. However, the association was not linear across the FR groups assessed (Figure 1).

The clinical and laboratory parameters were also compared between the two pregnant and non-pregnant groups (Table IV). No statistically significant differences in the parameters between the groups were found.

Discussion

Due to the potential importance of FF in fertilisation, this study investigated the hypothesis that the phospholipid level of FF would be correlated with fertility rates in a sample of women undergoing IVF/ICSI. This study provides the first report of the

Table II. Association between parameters and number of mature oocytes in patients undergoing IVF/ICS.

	r	*p* value
Age (years)	−0.21	0.04
Body mass index (kg/m^2)	0.03	0.74
ApoA-I (mg/dl)	−0.03	0.77
Total phospholipid (mg/dl)	0.18	0.12
Total phospholipid/apoA-I	0.09	0.46

r, Correlation coefficient; apoA-I, apolipoprotein A-I.

Table III. Association between parameters and fertilisation rate (FR) in patients undergoing IVF.

Parameter	*r*	*p* value
Age (years)	−0.09	0.44
Body mass index (kg/m^2)	−0.27	0.02
Sperm count (10^6/ml)	0.23	0.05
Sperm motility (%)	0.27	0.05
ApoA-I (mg/dl)	0.11	0.33
Total phospholipid (mg/dl)	−0.24	0.04
Total phospholipid/apoA-I	−0.24	0.04

FR, fertilisation rate. *r*; Correlation coefficient; apoA-I, apolipoprotein A-I. FR = (number of fertilised oocyte/number of mature oocytes) × 100.

Table IV. Level of follicular fluid parameters in clinical positive and negative fertility.

	Non-pregnant ($n = 76$)	Pregnant ($n = 24$)	*p* value
Age (years)	31.8 ± 5.6	31.2 ± 5.1	0.63
Body mass index (kg/m^2)	25.5 ± 3.2	25.7 ± 3.1	0.74
ApoA-I (mg/dl)	103 ± 20	106 ± 15	0.66
Total phospholipid (mg/dl)	34.5 ± 5.9	34.1 ± 7.4	0.84
Total phospholipid/apoA-I	0.35 ± 0.16	0.31 ± 0.07	0.25

Values are expressed as mean ± SD. Biochemical pregnancy was assessed by β-hCG test, 14 days after embryo transfer; apoA-I, apolipoprotein A-I.

possible effect of FF phospholipid on IVF/ICSI success rate in different laboratory and clinical levels. According to our findings, the FF concentration of phospholipids was significantly and negatively correlated to the fertility rate. This confirms the importance of lipid content in the FF for the fertility and health of women.

The findings regarding inverse correlation of age with number of mature oocytes and overall fertility rate in the studied population have formerly been discussed (Shaaker et al. 2012).

Fertility is critically dependent on a direct interaction between sperm and oocyte, and stimulation of phospholipid membrane fusion (Petcoff et al. 2008). The level of phospholipid in FF can modulate this process either by altering oocytes maturation rate or possibly through affecting surface phospholipid content of oocytes. The phospholipid content of FF is influenced by nutrition and metabolic condition of an individual. On the other hand, altered state of phospholipid metabolism is linked to several diseases such as pancreatitis, cancer, diabetes and coronary diseases (Gimenez et al. 2013). Interestingly, the plasma level of phospholipid has been linked to acute coronary syndromes (Cavusoglu et al. 2007) and diabetes (Hergenc et al. 2008) in population-based studies. A study by Huang and co-workers (2013) reported increased contents of phospholipid in the placenta of women with pre-eclampsia compared with normotensive controls. This increase has been proposed as a central mechanism of oxidative injury damage within the placental parenchyma and pathophysiology in pre-eclampsia. It has also been shown that apoA-I protects oocytes from oxidative damage and promotes fertility potential (Von et al. 2010). Therefore, elevated levels of phospholipid and decreased levels of apoA-I may contribute to increased oxidative stress in FF, causing decreased fertility. The present study supports this concept, because we have observed that the association of total phospholipids remains significant, even with the introduction of apoA-I. As the phospholipid/apoA-I in FF negatively correlated with fertility rate.

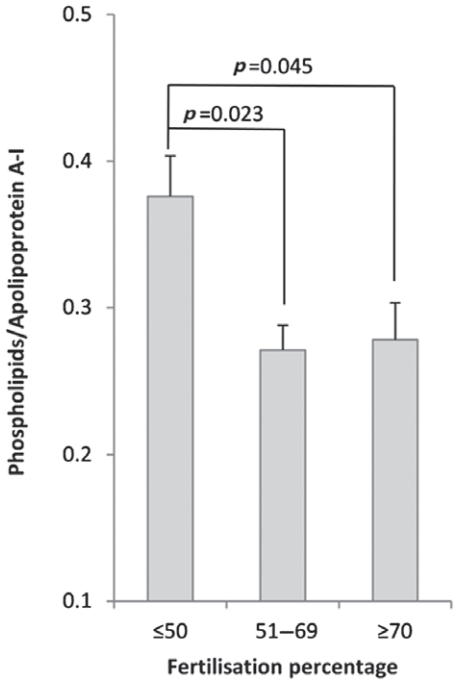

Figure 1. Levels of phospholipid/apolipoprotein A-I ratio in subgroups with various percentage of fertilisation. Multivariate analysis of variance with *post hoc* pair-wise comparison; *p* value adjusted for BMI, age, total phospholipid, sperm count and sperm motility.

In the current study, BMI and the level of phospholipid in FF showed significant inverse correlations with the percentage of fertilised oocytes. Consistent with our present findings, it has been shown that BMI correlated inversely with female fertility (Diamanti-Kandarakis and Bergiele 2001) and live-birth rate (Thum et al. 2007). It has also been evidenced that there is a direct relation between serum total phospholipid and BMI (Cavusoglu et al. 2007; Etherton and Kris-Etherton 1980). Thus, increased FF phospholipid may be a condition that affects fertility behind the obesity.

Fournier et al. (1996) have shown that the level of HDL phospholipid is directly correlated with cholesterol efflux potential of plasma. This phenomenon may also affect oocyte maturation and competence. Consistently, it has previously been observed that cholesteryl ester transfer protein (CETP) in FF was correlated positively with the maturity and the percentage of oocyte fertilisation (Mehdizadeh et al. 2011). CETP is a hydrophobic glycoprotein that facilitates cholesterol efflux, and its activity is affected by the phospholipid content of HDL particles (Rye et al. 2002). Our findings showed that phospholipid/apoA-I ratio had a negative correlation with the fertilisation rate. Additionally, there also appears to be a threshold effect, with those FR ≤ 50 having a much increased phospholipid/apoA-I. It seems that there is an interaction effect between FF phospholipid and apoA-I metabolism on oocyte maturation and female fertility status.

There were no significant correlation between measured variables and pregnancy rate. The occurrence of pregnancy is possibly affected by factors beyond the characteristics of FF, such as environmental stress, lifestyle and xenobiotics. Thus, the absence of a significant relationship between the parameters and the occurrence of pregnancy is probably because of the indirect nature of the relationship and/or the low number of subjects.

In this report, we focussed only on cases with no history of inflammatory or endocrine disorders. However, large prospective studies and interventional studies will be necessary to further elucidate the role of phospholipid on this subject. It remained to be clarified what mechanism is involved in the

change of fertility rate mediated by altered level of FF phospholipid, especially in patients undergoing IVF/ICSI. These could provide additional insight into the infertility pathogenesis and management.

In conclusion, while no association was found for pregnancy in the present study, the amounts of phospholipid and phospholipid/apoA-I ratio in FF were associated negatively to the percentage of oocyte fertilisation. Therefore, the change in the phospholipid and phospholipid/apoA-I ratio of FF might be regarded as indicators of female fertility.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

The research was partially supported by grants from the Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences and Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences.

References

Ali A, Virirak KT, Benkhalifa M, Miron P. 2007. Essential role of platelet-activating factor in male reproduction: a review. Reproductive Biomedicine Online 14:250–255.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911–917.

Buckingham KL, Stone PR, Smith JF, Chamley LW. 2006. Antiphospholipid antibodies in serum and follicular fluid – is there a correlation with IVF implantation failure? Human Reproduction 21:728–734.

Cavusoglu E, Chhabra S, Jiang XC, Hojjati MR, Chopra V, Eng C et al. 2007. Relation of baseline plasma phospholipid levels to cardiovascular outcomes at two years in men with acute coronary syndrome referred for coronary angiography. American Journal of Cardiology 100:1739–1743.

Chen PS, Toribara T Y, Warner H. 1956. Microdetermination of phosphorus. Analytical Chemistry 28:1756–1758.

Choi DH, Lee WS, Won M, Park M, Park HO, Kim E et al. 2010. The apolipoprotein A-I level is downregulated in the granulosa cells of patients with polycystic ovary syndrome and affects steroidogenesis. Journal of Proteome Research 9:4329–4336.

Diamanti-Kandarakis E, Bergiele A. 2001. The influence of obesity on hyperandrogenism and infertility in the female. Obesity Reviews 2: 231–238.

Etherton TD, Kris-Etherton PM. 1980. Characterization of plasma lipoproteins in swine with different propensities for obesity. Lipids 15:823–829.

Fournier N, de la Llera MM, Burkey BF, Swaney JB, Paterniti J Jr., Moatti N et al. 1996. Role of HDL phospholipid in efflux of cell cholesterol to whole serum: studies with human apoA-I transgenic rats. Journal of Lipid Research 37:1704–1711.

Fujimoto VY, Kane JP, Ishida BY, Bloom MS, Browne RW. 2010. High-density lipoprotein metabolism and the human embryo. Human Reproduction Update 16:20–38.

Gimenez MS, Oliveros LB, Gomez NN. 2013. Nutritional Deficiencies and Phospholipid Metabolism. International Journal of Molecular Sciences 12:2408–2433.

Hergenc G, Onat A, Sari I, Yazici M, Eryonucu B, Can G. 2008. Serum total and high-density lipoprotein phospholipid levels in a population-based study and relationship to risk of metabolic syndrome and coronary disease. Angiology 59:26–35.

Huang X, Jain A, Baumann M, Korner M, Surbek D, Butikofer P et al. 2013. Increased placental phospholipid levels in pre-eclamptic pregnancies. International Journal of Molecular Sciences 14:3487–3499.

Jakab A, Kovacs T, Zavaczki Z, Borsos A, Bray-Ward P, Ward D et al. 2003. Efficacy of the swim-up method in eliminating sperm with diminished maturity and aneuploidy. Human Reproduction 18: 1481–1488.

Kallen B. 2008. Maternal morbidity and mortality in in-vitro fertilization. Best Practice and Research. Clinical Obstetrics and Gynaecology 22: 549–558.

Mannock DA, Lewis RN, McMullen TP, McElhaney RN. 2010. The effect of variations in phospholipid and sterol structure on the nature of lipid-sterol interactions in lipid bilayer model membranes. Chemistry and Physics of Lipids 163:403–448.

Mehdizadeh A, Rahimipour A, Farzadi L, Darabi M, Vatankhah A, Golmohamadi Z et al. 2011. Correlation between the level of cholesteryl ester transfer protein in follicular fluid with fertilization rates in IVF/ICSI cycles. Iranian Journal of Reproductive Medicine 9:193–198.

Petcoff DW, Holland WL, Stith BJ. 2008. Lipid levels in sperm, eggs, and during fertilization in Xenopus laevis. Journal of Lipid Research 49: 2365–2378.

Rye KA, Duong M, Psaltis MK, Curtiss LK, Bonnet DJ, Stocker R et al. 2002. Evidence that phospholipids play a key role in pre-beta apoA-I formation and high-density lipoprotein remodeling. Biochemistry 41:12538–12545.

Sato H, Isogai Y, Masuda S, Taketomi Y, Miki Y, Kamei D et al. 2011. Physiological roles of group X-secreted phospholipase A2 in reproduction, gastrointestinal phospholipid digestion, and neuronal function. Journal of Biological Chemistry 286:11632–11648.

Shaaker M, Rahimipour A, Nouri M, Khanaki K, Darabi M, Farzadi L et al. 2012. Fatty acid composition of human follicular fluid phospholipids and fertilization rate in assisted reproductive techniques. Iranian Biomedical Journal 16:162–168.

Somfai T, Inaba Y, Watanabe S, Geshi M, Nagai T. 2012. Follicular fluid supplementation during in vitro maturation promotes sperm penetration in bovine oocytes by enhancing cumulus expansion and increasing mitochondrial activity in oocytes. Reproduction, Fertility, and Development 24:743–752.

Son WY, Chung JT, Das M, Buckett W, Demirtas E, Holzer H. 2013. Fertilization, embryo development, and clinical outcome of immature oocytes obtained from natural cycle in vitro fertilization. Journal of Assisted Reproduction and Genetics 30:43–47.

Thum MY, El-Sheikhah A, Faris R, Parikh J, Wren M, Ogunyemi T et al. 2007. The influence of body mass index to in-vitro fertilisation treatment outcome, risk of miscarriage and pregnancy outcome. Journal of Obstetrics and Gynaecology 27:699–702.

Tokumura A, Miyake M, Nishioka Y, Yamano S, Aono T, Fukuzawa K. 1999. Production of lysophosphatidic acids by lysophospholipase D in human follicular fluids of In vitro fertilization patients. Biology of Reproduction 61:195–199.

Von WT, Monisova Y, Hacker MR, Yoo SW, Penzias AS, Reindollar RR et al. 2010. Age-related variations in follicular apolipoproteins may influence human oocyte maturation and fertility potential. Fertility and Sterility 93:2354–2361.

Wallace M, Cottell E, Gibney MJ, McAuliffe FM, Wingfield M, Brennan L. 2012. An investigation into the relationship between the metabolic profile of follicular fluid, oocyte developmental potential, and implantation outcome. Fertility and Sterility 97:1078–1084.

Xu J, Kruger B, Vernunft A, Lohrke B, Viergutz T. 2009. Platelet-activating factor-stimulated production of reactive oxygen species in ovarian granulosa cells from periovulatory follicles. Cytometry 75A:658–664.