

Hypothesis regarding the effects of gonadotropins on the level of free fatty acids and phospholipids in serum and follicular fluid during controlled ovarian stimulation

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ABSTRACT

Controlled ovarian stimulation (COS) is used to augment the number of retrieved oocytes in *in vitro* fertilization (IVF). Follicular fluid (FF) contributes significantly to oocyte quality. Since the FF is composed of follicular secretions and plasma exudation, it reflects alterations in granulosa and thecal cells secretion as well as changes in the level of plasma constituents. Phospholipids (PL) and free fatty acids (FFA) are important constituents of both, FF and serum. Our hypothesis is that COS affects the level of PL and FFA in serum. Furthermore, since the level of PL and FFA in FF partially depends on their levels in serum, as a collateral of our hypothesis is that the existing level of PL and FFA in serum correlates with the levels of PL and FFA in FF, and that the dose of applied gonadotropins during COS will correlate with the levels of PL and FFA in serum and FF. In addition, we assume that the level of PL and FFA in serum and in FF after COS will correlate with the retrieved number of GQ oocytes, one of the most important outcomes of COS.

Introduction

Controlled ovarian stimulation (COS) is medically induced growth and maturation of multiple follicles and oocytes, in order to achieve as many as possible good quality (GQ) oocytes used in *in vitro* fertilization (IVF) [1]. Follicular fluid (FF), as the immediate environment of ovum, contributes significantly to oocyte maturation and quality [2]. Since the FF is composed of follicular secretions and plasma exudation, it reflects both alterations in the secretory processes of granulosa and thecal cells, and changes in the level of plasma constituents [3]. Therefore, contemporary research is focused on the influence of COS on serum and FF components and on the discovery of those biomarkers in FF and serum that could predict some important outcomes of COS, such as the number of GQ oocytes.

Phospholipids (PL) and free fatty acids (FFA) are important constituents of FF and serum [4]. In addition to being a major component of all cell membranes, PL play a number of important roles in

reproduction processes of both animals [5] and humans [6]. The importance of FFA for the reproductive system is reflected in the energy potential they possess, since the reproduction is an energetically expensive process [7]. Alterations in lipid profile can impact the human cumulus–oocyte complex (COC) during its maturation and increased follicular FFA have been associated with poor COC morphology [8].

Estrogens influence the metabolism of lipids by regulating the lipid metabolism in adipocytes and hepatocytes and by central regulation of lipid metabolism and therefore modulate the concentration of lipid substances in serum and FF [9,10]. In humans, estrogens have direct effects on lipolysis in adipocytes and estrogen deficiency is associated with increased levels of plasma FFA [11]. Furthermore, estrogen restrains accumulation of white adipose tissue by decreasing FFA and triglyceride (TG) synthesis and lipogenesis by repressing gene expression of lipoprotein lipase (LPL), regulating enzyme that disassembles plasma TG into FFA and glycerol [12].

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Hypothesis

Since COS increases estrogen levels and stimulates production of multiple ovarian follicles, estrogen levels in serum are higher in women undergoing COS during IVF procedure compared with estrogen levels found in healthy women during menstrual cycle or women using monophasic oral contraceptives [13,14]. COS represents rudimentary treatment for superovulation induction in assisted reproductive technology and IVF processes and it comprises the use of gonadotropins, which are influencing the composition, growth and maturation of follicles and FF and consequently estrogen levels [3]. COS could be a challenge for lipid metabolism, that could result in an excessive increase of serum PL and FFA and therefore could affect the reproduction processes. Thus, we hypothesized that serum levels of PL and FFA are modified/affected by gonadotropins used in COS. Furthermore, since the level of PL and FFA in FF partially depends on their levels in serum, as a collaterally of our hypothesis is that the existing level of PL and FFA in serum after COS correlates with the levels of PL and FFA in FF, and that the dose of applied gonadotropins during COS will correlate with the levels of PL and FFA in serum and FF. In addition, we assume that the level of PL and FFA in serum and in FF after COS will correlate with the retrieved number of GQ oocytes, one of the most important outcomes of COS (Fig. 1). Therefore, the hypothesis of our study is that serum levels of PL and FFA after COS could be valuable predictors of the oocytes quality and of the possible number of GQ oocytes achieved by COS. The purpose of this study is to identify potential biomarkers of oocyte quality, such as PL and FFA, that could be used for future investigations regarding the management of COS protocols in order to better predict the COS outcome in current assisted reproduction programs.

Patients and methods

This pilot study was conducted at the IVF Department of Clinic for Gynecology and Obstetrics “Narodni front”. This study included 20 infertile women participants undergoing IVF and the characteristics of the study population are presented in the Table 1. Regarding infertility cause, two women had PCOS, five women had PCOS and their male partners had abnormal semen analysis according to WHO criteria [15], 13 women were without identified infertility cause and 6 of them had partners with abnormal semen analysis and 7 with normal semen analysis. Women with BMI over 30, more than 40 years of age, with chronic cardiovascular, metabolic, kidney, hepatic, malignant and infective diseases were excluded. In order to determine the serum levels

of total FFA and total PL, blood samples were collected from all study participants immediately before the initiation of COS and after the completion of COS, on the day of oocyte retrieval. Follicular fluid samples for the assessment of total FFA and PL levels were collected from all patients instantly after the ovum pickup and the extraction of oocytes from FF. Baseline serum estradiol (E2) levels (on day 3 of menstrual cycle which precedes the cycle with COS) were measured in all participants. Furthermore, serum levels of E2 were measured immediately before the initiation and after the completion of COS on all study participants (Table 1). The study was approved by the Institutional Review Board (decision No. 05006-2017-7333) and an informed consent for biochemical and hormonal analysis of blood and follicular fluid was obtained from all study participants.

Gonadotropin-releasing hormone (GnRH) agonist (GnRH-a), and GnRH antagonist (GnRH-an) protocols were used for COS. The protocol type was chosen for each patient individually, according to the benefits and shortcomings of each treatment option, and more considerably on the patients' response and characteristics. Usually, GnRH-an protocol was used for the patients at high risk of developing ovarian hyperstimulation syndrome, patients with a higher number of previously unfavorable cycles, and patients at an advanced age. The presence of at least three follicles with diameter > 17 mm measured during transvaginal ultrasound scan was the criterion for triggering. In case of poor ovarian response, final oocyte maturation was induced if there had been only one follicle with diameter > 17 mm. In all protocols 10,000 IU of human chorionic gonadotropin (Pregnyl, Merck Sharp & Dohme d.o.o) were administered as an intramuscular bolus injection for ovulation triggering. Ultrasound guided aspirations were performed 35 h after triggering. The analyzed parameter of COS was the total number of GQ oocytes obtained by aspiration of follicles. Metaphase II oocytes and fertilized oocytes after IVF were considered to be GQ oocytes.

Measurements of serum E2 levels were performed in Laboratory Department of Clinic for Gynecology and Obstetrics “Narodni front” by specific chemiluminescence assay using the automated Elecsys immunoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were 0.5% and 0.1% and the results are expressed as pmol/l.

Serum and FF measurements of PL and FFA were performed in Laboratory of Radiobiology and Molecular Genetics at Institute Vinca, Belgrade, Serbia. Concentrations of PL in the serum and in FF were determined according to the colorimetric method described by Stewart [16]. The Stewart assay is based on the ability of the extracted phospholipids, in the presence of reagent 1 (aqueous solution of ferric chloride hexahydrate $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and ammonium thiocyanate NH_4SCN), to form a complex with ammonium ferric thiocyanate, with maximum of absorbance at 485 nm. Briefly, serum and FF samples (100 μl) were mixed with the mixture of chloroform/methanol (200 μl) in the ratio of 2:1. The resulting mixture was shaken for 20 s and then centrifuged for 3 min at 1000g. Up to 500 μl chloroform and then 500 μl of reagent 1 were added to the lower (chloroform) layer with extracted phospholipids. The resulting mixture was shaken for 20 s and then centrifuged for 10 min at 300g. After removing the upper layer, all sample and standard absorbance were read on a spectrophotometer (Lambda 35UV/VIS, Perkin Elmer, Waltham Massachusetts) at 485 nm and the phospholipids concentration was calculated from a phosphatidylcholine standard curve and expressed as mg/ml. Concentrations of FFA in the serum and FF were determined by a modified version of the Duncombe's method [17]. The principle of the method is based on the fact that the extracted FFA in chloroform, in the presence of an appropriate reagent (aqueous solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ with triethanolamine (TEA), pH 7.8), form copper salts, which in contact with diethyldithiocarbamate (DDC) builds a yellow complex compound with a maximum absorbance at 436 nm. The sample and standard absorbance were read on a spectrophotometer (Lambda 35UV/VIS, Perkin Elmer, Waltham Massachusetts) and the FFA concentrations were

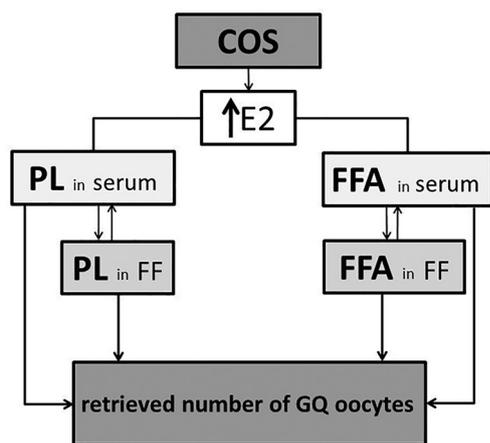


Fig. 1. The proposed mechanism of the effect of COS on the level of PL and FFA in serum and in FF and consequent correlation of PL and FFA levels in serum and in FF with the number of retrieved GQ oocytes. COS – controlled ovarian stimulation; ↑E2 – increase of estradiol; GQ – good quality.

Table 1
Anthropometric, hormonal and IVF characteristics of study participants.

Parameters	Mean ± SD	Min–max	p value
Age (years)	32.3 ± 3.3	27–40	NA
Body Height (cm)	168.1 ± 6.1	156–180	NA
Body Mass (kg)	64.6 ± 8.1	48–79.1	NA
BMI (kg/m ²)	22.72 ± 2.6	18.3–26.7	NA
Baseline serum E2 [pmol/l]	152.6 ± 16.8	41.2–289.9	NA
Serum E2 before COS [pmol/l]	67.1 ± 34.3	37–151	< 0.001***
Serum E2 after COS [pmol/l]	9747.1 ± 2535.9	3281–13,211	< 0.001***
Gonadotropin dose [IU]	1972.5 ± 501	1200–3000	NA
Number of retrieved oocytes	9.4 ± 2.9	4–14	NA
Number of retrieved GQ oocytes	6.6 ± 2.7	1–11	NA

p < 0.001*** – to baseline E2; p < 0.001### – to E2 before COS; NA – non applicable; BMI – body mass index; E2 – estradiol; COS – controlled ovarian stimulation; GQ – good quality.

calculated from a palmitate standard curve and expressed as mmol/l.

The Statistical Package for the Social Sciences (SPSS) 12.0 (SPSS Inc., Chicago, Illinois) was used for all statistical calculations. Data are presented as mean ± standard error (SE). Differences between each serum levels of PL and FFA before and after COS and between serum and FF levels of PL and FFA were analyzed by Student's *t*-test. Linear correlation analysis (Pearson) was used to test correlations between changes of used gonadotropin dose with PL and FFA levels in the serum after COS and in FF and between number of GQ oocytes and PL and FFA levels in the serum after COS and in FF. A *p* < 0.05 (2-tailed) was considered significant.

Preliminary results

Gonadotropin stimulation increases estrogen levels and stimulates production of multiple ovarian follicles. Serum estrogen levels are higher in women undergoing COS during IVF procedure compared with estrogen levels found in healthy women during menstrual cycle [13,14]. In our study, after COS, on the day of the follicles aspiration, serum E2 levels were increased for 14.6-fold compared with the serum E2 levels before the stimulation (Serum E2 before COS = 67.1 ± 34.3, Serum E2 after COS = 9747.1 ± 2535.9 in pmol/l, *p* < 0.001) (Table 1).

The assumption that gonadotropins, GnRH α and GnRH α n applied during COS affects lipid metabolic milieu and lipid levels in the serum and FF have been tested only in animal and *in vitro* experimental models until now [18–20]. GnRH α stimulates the metabolism of some PL in rats [18], results from experimental study performed in the culture of human preantral and preovulatory granulosa cells treated with GnRH α show increased secretion of PL [19]. The evaluation of serum level of PL in a healthy woman during different phases of menstrual cycle (early proliferative, late proliferative, mid-cycle, early luteal and late luteal phase) did not demonstrate significant differences [21], or significant decrease of PL levels was only found in the late luteal phase [22].

The results of our study support our hypothesis that COS affects blood levels of PL in humans. Our preliminary results (Fig. 2) show that the serum level of PL was increased after COS (*p* < 0.05). Significant difference was observed between the level of PL in FF and the level of PL in the serum before COS, with the level of PL being higher in FF (S1 = 100, FF = 133.92 ± 20.32%; *p* < 0.05). These results are in accordance with the studies performed in cows where COS modulated the PL content of bovine serum and FF with a significant increase in phosphatidylcholine 34:2 [20]. Increased levels of PL in serum, besides the impact of COS on estrogen levels and consequently on PL metabolism, could also be explained by enlarged needs for PL during the IVF procedure compared to natural menstrual cycle. Based on our results we suggest that COS results in great morphological changes of ovaries (increase in number of mature follicles leads to the alterations of weight and histomorphology in the ovaries) and endometrium [23], which caused increased processes of membranogenesis and consequently

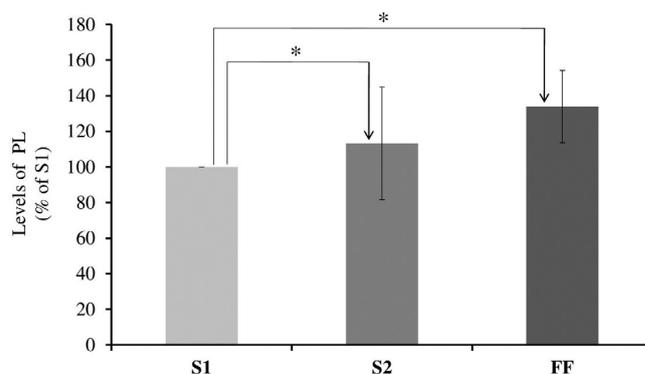


Fig. 2. Levels of phospholipids (PL) in serum before controlled ovarian stimulation (COS) (S1), after COS (S2) and in follicular fluid (FF) on the day of ovum pickup. The results are expressed in percentages (%) and represent the mean value ± SEM (n = 12–14); (* *p* < 0.05).

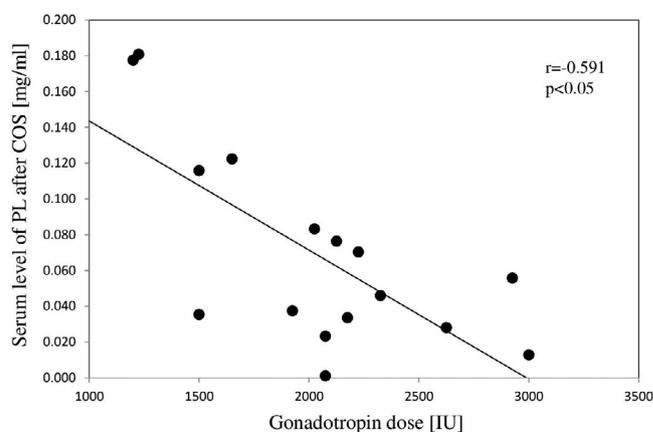


Fig. 3. Correlation between applied gonadotropin dose during controlled ovarian stimulation (COS) and serum level of phospholipids (PL) after COS. *r* – indicates Pearson correlation coefficient.

higher demands for PL [24]. This is additionally supported by a significant negative correlation (*p* < 0.05) observed in our study between the applied dose of gonadotropins and serum levels of PL after COS (Fig. 3), while our results show also that gonadotropin doses did not correlate with the levels of PL in FF. Women with good ovarian reserve (so-called “good responders” in terms of response to COS) have a significantly higher number of developing follicles and therefore intensified membranogenesis compared to women with poor ovarian reserve, so-called “poor responders” [25]. At the same time, “good responders” require lower doses of gonadotropins during COS compared to “poor responders” [25].

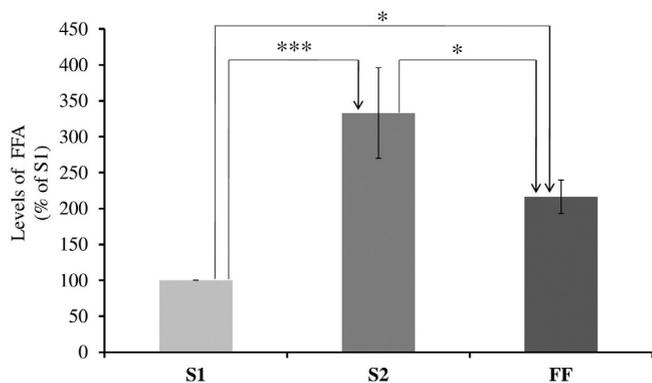


Fig. 4. Levels of total serum free fatty acids (FFA) before controlled ovarian stimulation (COS) (S1), after COS (S2) and in follicular fluid (FF) on the day of ovum pickup. The results are expressed in percentages (%) and represent the mean value \pm SEM (n = 14–16); (* p < 0.05 and *** p < 0.001).

Although, most serum metabolites, including PL and FFA, are reflected in human FF, they are present in FF at lower levels compared with serum [26] and this is inconsistent with our results regarding PL. We did not observe any significant difference between the level of PL in serum after COS and the level of PL in FF on the day of oocyte retrieval (Fig. 2). However, intensified synthesis of some types of PL that occurs in COC cells during IVF process [27] and the fact that our preliminary results were not BMI-related could explain disagreement between our results and the results demonstrated by Valckx et al. [26].

Correlation between serum and FF levels of PL and the number of retrieved GQ oocytes was not observed in our pilot study while Fayezi et al. found a significant negative correlation between the levels of PL in FF and fertilization ratio in women with wide range of infertility causes [28]. Participants in our study were women with unexplained infertility cause or PCOS. Therefore, we speculate that some other conditions underlying women's infertility (different than PCOS or unexplained infertility) could contribute or modulate the correlation between serum and FF levels of PL and the number of retrieved GQ oocytes.

Furthermore, our preliminary results show (Fig. 4) an increase of 232% in the serum level of FFA after COS, compared to the serum level of FFA before COS (p < 0.001). However, cyclic changes in E2 and progesterone production which occur during the normal menstrual cycle appear to have no effect on FFA mobilization [29]. FFA play an important role in the growth and maturation of oocyte, since β -oxidation of FFA provides energy for the growth and maturation of oocyte [30] and interplay of oocyte and COC cells has an important role. COC cells are rich in lipid droplets which contain TG. The TG are degraded in FFA and FFA are taken over by oocytes and used for oocytes metabolism [6]. Thus, monitoring the alterations of FFA levels in immediate surrounding of the oocyte, such as FF, could be important in the estimation of oocyte maturation and competence in IVF processes. FFA are incorporated in PL used for the intensified processes of membranogenesis and energy consumption, which are taking place in the multiple follicles development and maturation during COS [4]. As COS is a challenge for metabolism of FFA, it is clear that increased needs for FFA at the level of ovary results in higher levels of FFA in serum [4] and could explain the differences between FFA levels in serum between COS milieu and normal menstrual cycle.

Even essential substances have a negative impact on physiological processes if they excessively increase their levels. Since serum levels of FFA are increased in obesity, type 2 diabetes [31], and PCOS [32] all related to suboptimal reproductive outcomes and decreased fertility [33], we could expect that markedly increased serum levels of FFA adversely influence some IVF outcomes, such as the number of retrieved GQ oocytes and this assumption is supported by our preliminary results as a significant negative correlation (p < 0.05) between the serum level of FFA after COS and the number of retrieved GQ oocytes

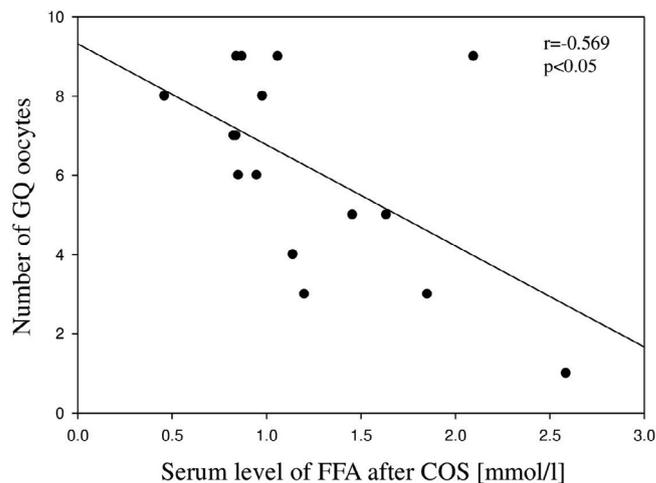


Fig. 5. Correlation between serum level of free fatty acids (FFA) after controlled ovarian stimulation (COS) and number of good quality (GQ) oocytes. r – indicates Pearson correlation coefficient.

have been observed (Fig. 5). These results could be because of a significant correlation between concentrations of FFA in serum and FF and association between elevated total follicular FFA levels and poorer COC quality observed in a study of Jungheim et al. [8] suggesting that excess of FFA after COS adversely influence ovarian follicular function. As a possible explanation for this effect could be the influence of FFA metabolism on Peroxisome proliferator-activated receptor gamma, an important enzyme in oocyte development [34,35] or alternatively via the inhibitory effects on important lipogenic pathways in granulosa cell function [36]. Furthermore, when serum concentrations of FFA exceed certain level, FFA are taken over by ovarian somatic cells, reducing their viability and steroidogenic capacity and inducing suppression of human granulosa cell proliferation and cell death via apoptosis in ovarian granulosa cells [37].

Our preliminary results show a higher level of FFA was observed in FF compared with the serum level of FFA before COS (p < 0.05) whereas the level of FFA in FF was lower (p < 0.05), compared with the serum level of FFA after COS (Fig. 4), and this is in line with the results of Valckx et al. [26]. The fact that FF faithfully reflects the content of FFA in serum, but not their levels, indicates that FFA are intensively used in follicle [2,26,30].

Based on the results from our study we suggest that the use of gonadotropins in the course of COS influences the serum PL and FFA levels, leading to their significant increase and therefore COS presents a kind of challenge for lipid metabolism. Besides, we demonstrated that the serum level of FFA after COS correlate with the number of GQ oocytes retrieved.

Conclusion

In conclusion, our preliminary data suggest that COS poses ability to challenge lipid metabolism and as a result of that increases E2, total FFA and total PL levels in serum. Furthermore, increased serum levels of FFA initiated by COS are negatively correlated with the number of retrieved GQ oocytes. Therefore, our assumption is that serum level of FFA after COS could be a good predictor of the number of retrieved GQ oocytes. On the other hand, this assumption could have a practical significance for future investigations regarding the management of COS protocols in current assisted reproduction programs. Serum level measurements of FFA after the completion of COS could be used in future research for monitoring the effects of modifications of COS protocols in order to achieve better COS outcomes, such as the number of obtained GQ oocytes.

Conflicts of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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References

- [1] Elder K, Dale B. *In vitro* fertilization. 2nd ed. Cambridge: Cambridge University Press; 2000.
- [2] Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod Biol Endocrinol* 2009;7:40.
- [3] Edwards RG. Follicular fluid. *J Reprod Fertil* 1974;37(1):189–219.
- [4] Shaaker M, Rahimpour A, Nouri M, et al. Fatty acid composition of human follicular fluid phospholipids and fertilization rate in assisted reproductive techniques. *Iran Biomed J* 2012;16(3):162–8.
- [5] Kobayashi T, Yamano S, Murayama S, Ishikawa H, Tokumura A, Aono T. Effect of lysophosphatidic acid on the preimplantation development of mouse embryos. *FEBS Lett* 1994;351(1):38–40.
- [6] Tokumura A, Miyake M, Nishioka Y, Yamano S, Aono T, Fukuzawa K. Production of lysophosphatidic acids by lysophospholipase D in human follicular fluids of *In vitro* fertilization patients. *Biol Reprod* 1999;61(1):195–9.
- [7] Dunning KR, Russell DL, Robker RL. Lipids and oocyte developmental competence: the role of fatty acids and beta-oxidation. *Reproduction* 2014;148(1):R15–27.
- [8] Jungheim ES, Macones GA, Odem RR, et al. Associations between free fatty acids, cumulus oocyte complex morphology and ovarian function during *in vitro* fertilization. *Fertil Steril* 2011;95(6):1970–4.
- [9] Szafran H, Smiela-Korombel W. The role of estrogens in hormonal regulation of lipid metabolism in women. *Przegl Lek* 1998;55(5):266–70.
- [10] Faulds MH, Zhao C, Dahlman-Wright K, Gustafsson JA. The diversity of sex steroid action: regulation of metabolism by estrogen signaling. *J Endocrinol* 2012;212(1):3–12.
- [11] Jensen MD, Martin ML, Cryer PE, Roust LR. Effects of estrogen on free fatty acid metabolism in humans. *Am J Physiol* 1994;266(6 Pt 1):E914–20.
- [12] Safe S, Kim K. Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. *J Mol Endocrinol* 2008;41(5):263–75.
- [13] Magnani B, Tsen L, Datta S, Bader A. *In vitro* fertilization. Do short-term changes in estrogen levels produce increased fibrinolysis? *Am J Clin Pathol* 1999;112(4):485–91.
- [14] Stening KD, Berg G, Hammar M, et al. Influence of estrogen levels on thermal perception, pain thresholds, and pain tolerance: studies on women undergoing *in vitro* fertilization. *J Pain* 2012;13(5):459–66.
- [15] WH Organization. WHO laboratory manual for the examination and processing of human semen. 2010.
- [16] Stewart JCM. Colorimetric determination of phospholipids with ammonium ferri-thiocyanate. *Anal Biochem* 1980;104(1):10–4.
- [17] Duncombe WG. The colorimetric micro-determination of long-chain fatty acids. *Biochem J* 1963;88(1):7–10.
- [18] Davis JS, Farese RV, Clark MR. Gonadotropin-releasing hormone (GnRH) stimulates phosphatidylinositol metabolism in rat granulosa cells: mechanism of action of GnRH. *Proc Natl Acad Sci USA* 1983;80(7):2049–53.
- [19] Naor Z, Yavin E. Gonadotropin-releasing hormone stimulates phospholipid labeling in cultured granulosa cells. *Endocrinology* 1982;111(5):1615–9.
- [20] Santos PH, Fontes PK, Franchi FF, et al. Lipid profiles of follicular fluid from cows submitted to ovarian superstimulation. *Theriogenology* 2017;94:64–70.
- [21] Punnonen R. Total serum cholesterol, triglycerides and phospholipids during the normal menstrual cycle. *Int J Gynaecol Obstet* 1978;15(4):296–8.
- [22] Ahumada Hemer H, Valles de Bourges V, Juarez Ayala J, Brito G, Diaz-Sanchez V, Garza-Flores J. Variations in serum lipids and lipoproteins throughout the menstrual cycle. *Fertil Steril* 1985;44(1):80–4.
- [23] Wang LY, Wang N, Le F, et al. Superovulation induced changes of lipid metabolism in ovaries and embryos and its probable mechanism. *PLoS One* 2015;10(7).
- [24] Montane JL, Perez-Ballester B. Cyclic changes in phospholipid content and composition in human endometrium during the menstrual cycle. *J Reprod Fertil* 1985;73(2):317–21.
- [25] Li HWR, Lee VCY, Ho PC, Ng EHY. Ovarian sensitivity index is a better measure of ovarian responsiveness to gonadotrophin stimulation than the number of oocytes during *in-vitro* fertilization treatment. *J Assist Reprod Genet* 2014;31(2):199–203.
- [26] Valckx SDM, De Pauw I, De Neubourg D, et al. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod* 2012;27(12):3531–9.
- [27] Gómez-Torres MJ, García EM, Guerrero J, et al. Metabolites involved in cellular communication among human cumulus-oocyte-complex and sperm during *in vitro* fertilization. *Reprod Biol Endocrinol* 2015;13(1):123.
- [28] Fayezi S, Darabi M, Darabi M, Nouri M, Rahimpour A, Mehdizadeh A. Analysis of follicular fluid total phospholipids in women undergoing *in-vitro* fertilisation. *J Obstet Gynaecol* 2014;34(3):259–62.
- [29] Heiling VJ, Jensen MD. Free fatty acid metabolism in the follicular and luteal phases of the menstrual cycle. *J Clin Endocrinol Metab* 1992;74(4):806–10.
- [30] Songsasen N. Energy metabolism regulating mammalian oocyte maturation. In: Swan A, editor. *Meiosis – molecular mechanisms and cytogenetic diversity*. 2012.
- [31] Boden G. Obesity, insulin resistance and free fatty acids. *Curr Opin Endocrinol Diabetes Obes* 2011;18(2):139–43.
- [32] Niu Z, Lin N, Gu R, Sun Y, Feng Y. Associations between insulin resistance, free fatty acids, and oocyte quality in polycystic ovary syndrome during *in vitro* fertilization. *J Clin Endocrinol Metab* 2014;99(11):E2269–76.
- [33] Legro RS. Obesity and PCOS: implications for diagnosis and treatment. *Semin Reprod Med* 2012;30(6):496–506.
- [34] Downs SM, Mosey JL, Klinger J. Fatty acid oxidation and meiotic resumption in mouse oocytes. *Mol Reprod Dev* 2009;76(9):844–53.
- [35] 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(5):2646–56.
- [36] Robker RL, Akison LK, Bennett BD, et al. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab* 2009;94(5):1533–40.
- [37] Mu YM, Yanase T, Nishi Y, et al. Saturated FFAs, palmitic acid and stearic acid, induce apoptosis in human granulosa cells. *Endocrinology* 2001;142(8):3590–7.