J Assist Reprod Genet (2015) 32:1637–1642
DOI 10.1007/s10815-015-0582-3

ASSISTED REPRODUCTION TECHNOLOGIES

The effect of serum and follicular fluid amyloid-associated protein levels on in vitro fertilization outcome in patients with polycystic ovary syndrome

Hakan Timur[1] · **Nafiye Yimaz**[1] · **Inci Kahyaoglu**[2] · **Hasan Ali Inal**[3] · **Salim Erkaya**[1]

Received: 5 July 2015 / Accepted: 23 September 2015 / Published online: 10 October 2015

Abstract
Purpose In this study, we aimed to investigate serum and follicular fluid amyloid A protein levels in non-obese non-hyperandrogenic patients with polycystic ovary syndrome (PCOS) undergoing in vitro fertilization (IVF) and IVF outcome.
Methods A total of 81 patients undergoing IVF treatment, 41 patients diagnosed as PCOS according to the Rotterdam criteria (group I) and 40 patients with the etiology of male factor infertility (group II), were included in the study. On the day of oocyte pickup, serum and follicular fluid samples were collected from all patients.
Results Serum E2 level on the day of hCG (2849.93±541.54 vs. 2494.28±712.98) and total number of retrieved oocytes (13.73±3.57 vs. 10.53±4.07) were significantly higher in group I when compared to group II ($p<0.05$). However, number of mature oocytes, fertilization rate, and clinical pregnancy rate did not differ ($p>0.05$). No significant difference was found between two groups regarding the serum and follicular fluid amyloid A protein levels on the day of oocyte retrieval ($p>0.05$).

Keywords Polycystic ovary syndrome · Amyloid A · In vitro fertilization · Adipokine

Capsule In a selected population of non-obese non-hyperandrogenic PCOS patients, no significant difference is detected between the study and the control group regarding the amyloid A levels despite the inflammatory nature of PCOS and IVF outcome.

✉ Hakan Timur
drhakantimur@gmail.com

[1] Department of Reproductive Endocrinology, Dr. Zekai Tahir Burak Women's Health Education and Research Hospital, ZTB, Hamaonu, Altindag, 06500 Ankara, Turkey

[2] Department of Reproductive Endocrinology, Etlik Zubeyde Hanim Women's Health Education and Research Hospital, Ankara, Turkey

[3] Department of Obstetrics and Gynecology, Konya Education and Research Hospital, Konya, Turkey

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5–10 % of women of reproductive age [1]. It is defined as a pro-inflammatory state characterized by menstrual irregularities and hyperandrogenism [2]. Patients with PCOS are usually obese (40–70 %) contributing to insulin resistance, dyslipidemia, and risk of cardiovascular disease [2, 3]. Adipose tissue is thought to play a key role in endocrine and metabolic control, creating a range of adipokines into the circulation, many of which increase inflammation [4]. Serum concentrations of high-sensitive C-reactive protein (hs-CRP), interleukin-18, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1a (MIP-1a) are found to be elevated in PCOS [5, 6].

Inflammatory proteins stimulate or inhibit cell growth, regulate cell differentiation, induce cell chemotaxis, and modulate the expression of other cytokines. Cytokine function in the ovary has been demonstrated as promoting processes of follicular growth, steroidogenesis, recruitment, and activation of leukocytes necessary for ovulation and tissue remodeling during ovulation, luteinization, and luteolysis [6]. Some of the cytokines such as interleukin (IL)-12 influence oocyte fertilization and embryo quality, while others, such as IL-1, IL-8, and IL-18 were found to be correlated successfully with pregnancy rate in IVF treatment [7–9]. On the other hand, tumor necrosis factor (TNF)-alpha elevation and higher CRP levels

may be correlated with an increased risk of IVF failure [10, 11].

Recently, serum amyloid-associated (AA) protein was proposed as an inflammatory marker that might be more specific to PCOS-related inflammation [9]. Few authors researched the relation between PCOS and serum AA protein documenting different results with increased serum levels in PCOS [2, 12, 13] or no difference with the controls [1]. Up to our knowledge, there is only one report in the literature studying the levels of AA protein in follicular fluid (FF) of women undergoing controlled ovarian stimulation for in vitro fertilization [14]. Therefore in this study, we aimed to investigate serum and follicular fluid levels of AA protein in non-hyperandrogenic, non-obese patients with PCOS.

Materials and methods

Study subjects and data collection

The study was performed in the assisted reproduction clinic of a tertiary research and education hospital between April 2014 and August 2014. A total of 81 patients, 41 non-obese, and non-hyperandrogenic patients diagnosed as PCOS according to the Rotterdam consisting the study group (group I) and 40 patients diagnosed as male factor infertility as the control group (group II), were recruited for the study. Exclusion criteria were as follows: (1) patients with the evidence of clinical hyperandrogenemia and (2) body mass index (BMI)> 30 kg/m^2. The exclusion criteria were designated in order to exclude the effects of BMI and insulin resistance on serum and FF AA levels [1, 12, 13]. This study was approved by the institutional review board of the hospital. Written informed consent was obtained from all patients.

In vitro fertilization stimulation

All patients underwent a GnRH agonist long luteal downregulation protocol with the administration of the GnRH agonist leuprolide acetate (Lucrin, Abbot, Turkey) in the midluteal phase of the previous cycle until the day of hCG administration. After the satisfactory pituitary desensitization was achieved (serum E2 level<50 pg/ml, endometrial thickness <5 mm, serum LH levels <5 IU/ml), GnRH agonist dose was reduced to half and recombinant FSH (Gonal-F; Merck Serono, Istanbul, Turkey or Puregon, Organon, Istanbul, Turkey) administration was started according to the patient's age, baseline serum FSH concentration on day 3, and body mass index (BMI). Adjustment of gonadotropin doses was done according to the ovarian response monitored by serial E2 measurements and transvaginal ultrasonography. Recombinant hCG (250 μg sc., Ovitrelle, Serono, Istanbul, Turkey) was administered when at least three follicles showed a mean diameter of 18 mm, and oocyte retrieval was done 36 h after the hCG injection by the guidance of transvaginal ultrasonography, and ICSI was used for all IVF-ET patients. Fresh single-embryo transfers performed on day 3 to all women [14]. Luteal support was given by vaginal progesterone (Crinone 8 % gel, Serono, Istanbul), and supplementation was started on hCG administration day [15]. Pregnancy was determined by βhCG levels in blood tests performed 14 days after embryo transfer, and clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat.

Collection of blood serum and follicular fluid samples

Follicular fluid was collected from the first follicle entered which is ≥18 mm in size and from which an oocyte was retrieved. Flushing was not performed. Samples contaminated with blood were excluded. Blood and FF samples of 81 patients were centrifuged for 15 min at 3000 rpm on the day of follicle puncture and stored at −80 °C during 5 months for subsequent analysis. Each sample was masked to be analyzed blindly.

Amyloid A assay in serum and follicular fluid

AA levels in serum and FF were measured with Serum Amyloid A (SAA), Human, ELISA Kit (Cloud-Clone Corp, USA) used for the quantitative detection of AA in serum, plasma, and other biological fluids. Detection range of the kit ranged between 0.312 and 20 ng/ml, with a sensitivity of 0.125 ng/ml. The calculated overall intraassay and interassay coefficient of variation was <10 and <12 %, respectively. The ELISA assay was performed according to the manufacturer's protocol and guideline.

Outcome measures

The primary outcome measures were serum and FF AA protein levels. The secondary outcome measures included number of mature oocytes, fertilization rate, embryo quality, the clinical pregnancy, and live birth rates.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software (15.0, SPSS Inc., Chicago, IL, USA). Shapiro–Wilk test was used to test the distribution of variables. Continuous variables were presented as mean±standard deviation values and compared using the independent samples *t* test and Mann–Whitney *U* test. Categorical variables were compared with Fisher's exact or Pearson chi-squared tests when available. Whether the potential risk factors have statistically significant affect on clinical pregnancy or not was evaluated by

univariate logistic regression analyses. Odds ratios and 95 % confidence intervals for each independent variable were also calculated. Statistical significance was assumed with a probability of 0.05.

Results

No difference was found between the two groups regarding age, BMI, and basal hormonal profile. Duration of stimulation and total dose of gonadotropins used were similar in both groups ($p>0.05$). Serum E2 level on the day of hCG (2849.93±541.54 vs. 2494.28±712) and total number of retrieved oocytes (13.73±3.57 vs. 10.53±4.07) were significantly higher in group I when compared to group II ($p<0.05$). However, number of mature oocytes, fertilization rate, and clinical pregnancy rate did not differ ($p>0.05$). No significant difference was found between the two groups regarding the serum and FF AA protein levels on the day of oocyte retrieval ($p>0.05$). Although there was no difference between the two groups regarding leukocyte count, neutrophil count (NC), neutrophil/leukocyte (N/L) ratio, and hs-CRP were significantly higher in group I than group II ($p<0.05$) (Table 1). There was no difference between the two groups regarding perinatal outcomes ($p>0.05$) (Table 2).

When we performed univariate logistic regression analysis, PCOS, age, BMI, day 3 FSH levels, embryo quality, number of transferred embryos, and serum AA protein levels do not affect on clinical pregnancy ($p>0.05$). But, we found that each 1 ng/ml decrease of FF AA protein levels increases the likelihood of clinical pregnancy as 1.479 times [(95 % CI) 1.479 (1.056–2.070)] ($p=0.023$) (Table 3).

Discussion

This is the first study in the literature studying both serum and FF levels of AA in lean PCOS patient undergoing controlled ovarian hyperstimulation. The results revealed no significant difference between PCOS and control groups regarding both the serum and FF AA protein levels. Although E2 levels on the hCG day and number of retrieved oocytes were significantly higher in the PCOS group, no difference was found between fertilization or clinical pregnancy rates. Neutrophil count, neutrophil/leukocyte ratio, and hs-CRP levels were found to be significantly higher in the PCOS group, indicating the inflammatory nature of the PCOS.

Polycystic ovary syndrome is associated with multiple metabolic abnormalities. Approximately 50 % of PCOS patients fulfill the criteria for having metabolic syndrome [16–18]. Patients usually have central obesity, insulin resistance, dyslipidemia, inflammation, and risk of type 2 diabetes [10, 19]. Fat accumulation on the trunk and in visceral depots has been reported in women with PCOS regardless of BMI [20].

Table 1 Controlled ovarian stimulation parameters, hematologic parameters, blood, and follicular fluid AA protein levels of the groups

	PCOS (group I) ($n=41$)	Control (group II) ($n=40$)	p Value
Age (years)	27.46±3.37	28.10±2.44	0.189
BMI (kg/m^2)	24.94±1.74	24.60±1.29	0.090
D3 FSH (IU/ml)	6.24±0.93	6.58±0.94	0.104
D3 E_2 (pg/ml)	43.86±11.48	40.30±12.68	0.188
Duration of stimulation (day)	9.49±0.81	9.85±1.18	0.112
Total gonadotropin dose (IU)	1585.37±380.00	1750.00±574.20	0.131
E_2 level on hCG day (pg/ml)	2849.93±541.54	2494.28±712.98	0.013*
Endometrial thickness on hCG day (mm)	9.96±1.01	10.35±1.29	0.135
Number of retrieved oocytes	13.73±3.57	10.53±4.07	0.001*
Number of mature oocytes	10.27±3.63	8.88±4.65	0.137
Fertilization rate (%)	50.35±16.61	56.79±20.82	0.127
Patients with grade 1 and 2 embryos (%)	15 (36.6 %)	18 (45.0 %)	0.441
Leukocyte count (10^3) (mcl)	6.82±1.01	6.39±1.38	0.114
Neutrophil count (10^3) (mcl)	4.33±0.82	3.56±1.10	0.001*
Neutrophil/leukocyte ratio (%)	63.96±9.86	56.89±17.45	0.029*
hs-CRP (mg/l)	11.96±1.65	8.48±1.55	0.001*
FF AA protein (ng/ml)	2.66±1.51	2.24±1.39	0.214
Serum AA protein (ng/ml)	0.23±0.04	0.21±0.04	0.105

PCOS polycystic ovary syndrome, *BMI* body mass index, *FSH* follicle-stimulating hormone, *E2* estradiol, *hCG* human chorionic gonadotropin, *hs-CRP* high-sensitive C-reactive protein, *FF* follicular fluid, *AA* amyloid associated

*Statistically significant

Table 2 Perinatal outcomes of the groups

	PCOS (group I) (n=41)	Control (group II) (n=40)	p Value
Clinical pregnancy rate (%)	13 (31.7 %)	15 (37.5 %)	0.584
Abortion rate (%)	3 (7.3 %)	2 (5 %)	0.664
Biochemical pregnancy rate (%)	1 (2.4 %)	2 (5 %)	0.538
Ongoing pregnancy rate (%)	9 (21.9 %)	11 (27.5 %)	0.562
Live birth rate (%)	9 (21.9 %)	10 (25 %)	0.746

$p<0.05$ is significant

The visceral adipocytes exert these effects in a paracrine and endocrine manner via the secretion of adipokines into the circulation, many of which increase inflammation [4, 6]. The link between inflammation and metabolic disorders was first recognized by Hotamisligil et al. who demonstrated that adipocytes express the pro-inflammatory cytokine TNF-α and TNF-α expression is markedly increased in adipocytes of obese mice [21]. Besides TNF-α, several other cytokines, like IL-6, was found to be secreted from adipose tissue [22]. Serum AA protein is another acute phase protein which is thought to be more specific to PCOS-related inflammation [23]. Serum AA protein is produced by the liver in response to inflammatory stimuli [24]. However, in the non-acute phase, adipose tissue may be the major source of serum AA protein [25], and serum AA messenger RNA (mRNA) expression is markedly higher in hypertrophic adipocytes [26]. Serum AA protein has been implicated in inflammation, insulin resistance, and atherosclerosis [4]. However, there are limited studies in the literature regarding the relationship between serum AA protein and PCOS revealing conflicting results [1]. Tan et al. reported that PCOS patients (mean BMI of patients >30 kg/m^2) had approximately twofold elevation in the serum AA protein levels when compared to age, BMI, and waist hip circumference (WHR) matched controls. Both subcuteneous and omental adipose tissues from the PCOS patients had approximately twofold elevation in AA mRNA expression as compared to control subjects, suggesting that the adipose tissue in PCOS patients may be the primary source of increased inflammation [2]. In other study by Blair et al., serum AA protein was found to be increased in the PCOS women, when matched with control women for BMI and IR and that this increase was independent of changes to other inflammatory markers, including hs-CRP, myeloperoxidase, and neopterin [13]. The results of the study by Gidwani et al. [12] also support the previous reports, demonstrating increased levels of serum AA protein in obese PCOS patients. On the other hand, Manneras-Holm reported that serum AA protein levels did not differ between 31 mildly overweight PCOS (BMI=24.8±4.8 kg/m^2) and 31 weight-matched controls [1].

There is limited data about the role of AA protein in ovarian physiology. Urieli-Shoval et al. [23] studied follicular fluid and serum levels of AA protein in IVF patients. They found a strong correlation between serum and FF levels and also reported a significant association between BMI and serum AA protein concentrations, that is higher levels associated with increased BMI. Besides obesity, controlled ovarian hyperstimulation, which induces a pro-inflammatory state [27], was indicated as second reason of elevated levels of serum AA protein [23]. In our study, although not statistically significant, both the levels of FF and serum AA protein levels of non-obese PCOS group revealed higher levels than control group. They also showed reduced pregnancy rates in elevated serum AA protein group when compared to normal levels (43 vs.

Table 3 Logistic regression analysis of the factors thought to be effective on clinical pregnancy

	Clinical pregnancy (−) (n=53)	Clinical pregnancy (+) (n=28)	p Value	OR (95 % CI)
PCOS	28 (52.8 %)	13 (46.4 %)	0.584	0.774 (0.309–1.938)
Age (years)	27.66±3.04	28.00±2.81	0.621	1.040 (0.890–1.215)
BMI (kg/m^2)	24.86±1.21	24.61±1.50	0.411	0.860 (0.601–1.232)
D3 FSH (IU/ml)	6.44±1.04	6.36±0.75	0.710	0.910 (0.556–1.492)
Patients with grade 1 and 2 embryos (%)	21 (39.6 %)	12 (42.9 %)	0.778	1.143 (0.451–2.894)
Number of transferred embryos	1.11±0.31	1.17±0.39	0.605	1.667 (0.241–11.525)
FF AA protein (ng/ml)[a]	2.99±1.66	2.17±1.27	**0.023**	1.479 (1.056–2.070)
Serum AA protein (ng/ml)[a]	0.23±0.049	0.22±0.037	0.400	109.582 (0.002–621×10^4)

OR odds ratio, *CI* confidence interval

[a] The effects of each 1 ng/ml decrease in AA protein levels

18.8 %). On the other hand, we could not demonstrate such a result. The role of AA protein in modulation of cholesterol metabolism and transport, thereby influencing ovarian steroidogenesis, may explain this relation in a sense [4, 28].

There are a lot of studies in the literature researching a relationship between PCOS and inflammatory markers. While some investigators demonstrated significant elevations in NC, WBC count, or hs-CRP [11, 29], others could not find increased inflammatory markers in PCOS patients undergoing IVF [30–32]. In our study, we demonstrated a significant difference between PCOS and control group regarding NC, neutrophil/leukocyte ratio, and also hs-CRP levels.

In conclusion, we could not demonstrate between the study and the control groups regarding serum and FF AA levels and also the relation between AA and good ovarian response (number of retrieved oocytes, E2 levels on hCG day) or clinical pregnancy rates. Further studies in a larger series are needed to determine this relation.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Manneras Holm L, Leonhardt H, Kullberg J, Jennische E, Oden A, Holm G, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. J Clin Endocrinol Metab. 2011;96:E304–11.
2. Tan BK, Adya R, Shan X, Aghilla M, Lehnert H, Keay SD, et al. The anti-atherogenic aspect of metformin treatment in insulin resistant women with the polycystic ovary syndrome: role of the newly established pro-inflammatory adipokine Acute-phase Serum Amyloid A; evidence of an adipose tissue-monocyte axis. Atherosclerosis. 2011;216:402–8.
3. Borruel S, Fernandez-Duran E, Alpanes M, Marti D, Alvarez-Blasco F, Luque Ramirez M, et al. Global adiposity and thickness of intraperitoneal and mesenteric adipose tissue depots are increased in women with polycystic ovary syndrome (PCOS). J Clin Endocrinol Metab. 2013;98:1254–63.
4. Yang RZ, Lee MJ, Hu H, Pollin TI, Ryan AS, Nicklas BJ, et al. Acute phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med. 2006;3:e287.
5. Escobar-Morreale HF, Luque-Ramírez M, González F. Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. Fertil Steril. 2011;95(3):1048–58.e1-2.
6. Duleba AJ, Dokras A. Is PCOS an inflammatory process? Fertil Steril. 2012;97:7–12.
7. Sarapik A, Velthut A, Haller-Kikkatalo K, Faure GC, Bene MC, de Carvalho Bittencourt M, et al. Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clin Dev Immunol. 2012;2012:606459. doi:10.1155/2012/606459.
8. Malizia BA, Wook YS, Penzias AS, Usheva A. The human ovarian follicular fluid level of interleukin-8 is associated with follicular size and patient age. Fertil Steril. 2010;93(2):537–43. doi:10.1016/j.fertnstert.2008.11.033.
9. Vujisic S, Lepej SZ, Emedi I, Bauman R, Remenar A, Tiljak MK. Ovarian follicular concentration of IL-12, IL-15, IL-18 and p40 subunit of IL-12 and IL-23. Hum Reprod. 2006;21(10):2650–5.
10. Winger EE, Reed JL, Ashoush S, El-Toukhy T, Taranissi M. Die-off ratio correlates with increased TNF-α:IL-10 ratio and decreased IVF success rates correctable with humira. Am J Reprod Immunol. 2012;68(5):428–37. doi:10.1111/j.1600-0897.2012.01179.x.
11. Levin I, Gamzu R, Mashiach R, Lessing JB, Amit A, Almog B. Higher Creactive protein levels during IVF stimulation are associated with ART failure. J Reprod Immunol. 2007;75(2):141–4.
12. Gidwani S, Phelan N, Mcgill J, Mcgowan A, O'Connor A, Young IS, et al. Polycystic ovary syndrome influences the level of serum amyloid A and activity of phospholipid transfer protein in HDL2 and HDL3. Hum Reprod. 2014;29:1518–25.
13. Blair SA, Kyaw-Tun T, Young IS, Phelan NA, Gibney J, Mceneny J. Oxidative stress and inflammation in lean and obese subjects with PCOS. J Reprod Med. 2013;58:107–14.
14. Berger DS, Zapantis A, Merhi Z, Younger J, Polotsky AJ, Jindal SK. Embryo quality but not pronuclear score is associated with clinical pregnancy following IVF. J Assist Reprod Genet. 2014;31(3):279–83. doi:10.1007/s10815-013-0162-3.
15. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database Syst Rev. 2015;7:CD009154. doi:10.1002/14651858.CD009154.pub3.
16. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33(6):981–1030. doi:10.1210/er.2011-1034.
17. Hojlund K. Metabolism and insulin signaling in common metabolic disorders and inherited insulin resistance. Dan Med J. 2014;61(7):B4890.
18. Alebic MS, Bulum T, Stojanović N, Duvnjak L. Definition of insulin resistance using the homeostasis model assessment (HOMA-IR) in IVF patients diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria. Endocrine. 2014;47(2):625–30. doi:10.1007/s12020-014-0182-5.
19. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:1929–35.
20. Carmina E, Bucchieri S, Esposito A, Del Puente A, Mansueto P, Orio F, et al. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab. 2007;92:2500–5.
21. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259:87–91.
22. Yap SH, Moshage HJ, Hazenberg BPC, Roelofs HM, Bijzet J, Limburg PC, et al. Tumor necrosis factor (Tnf) inhibits interleukin (Il)-1 and/or Il-6 stimulated synthesis of C-reactive protein (Crp) and serum amyloid a (Saa) in primary cultures of human hepatocytes. Biochim Biophys Acta. 1991;1091:405–8.
23. Urieli-Shoval S, Finci Yeheskel Z, Eldar I, Linke RP, Levin M, Prus D, et al. Serum amyloid a: expression throughout human ovarian folliculogenesis and levels in follicular fluid of women undergoing controlled ovarian stimulation. J Clin Endocrinol Metab. 2013;98:4970–8.
24. O'Brien KD, Chait A. Serum amyloid A: the "other" inflammatory protein. Curr Atheroscler Rep. 2006;8:62–8.
25. Sjoholm K, Palming J, Olofsson LE, Gummesson A, Svensson PA, Lystig TC, et al. A microarray search for genes predominantly expressed in human omental adipocytes: adipose tissue as a major production site of serum amyloid A. J Clin Endocrinol Metab. 2005;90:2233–9.
26. Jernas M, Palming J, Sjoholm K, Jennische E, Svensson PA, Gabrielsson BG, et al. Separation of human adipocytes by size:

hypertrphic fat cells display distinct gene expression. FASEB J. 2006;20:1540–42.
27. Orvieto R. Controlled ovarian hyperstimulation-an inflammatory state. J Soc Gynecol Investig. 2004;11:424–26.
28. Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute phase protein, in normal and disease states. Curr Opin Hematol. 2000;7:64–9.
29. Kahyaoglu I, Yilmaz N, Timur H, Inal HA, Erkaya S. Granulocyte colonystimulating factor: A relation between serum and follicular fluid levels and in-vitro fertilization outcome in patients with polycystic ovary syndrome. Cytokine. 2015;74(1):113–6.
30. Orvieto R, Chen R, Ashkenazi J, Ben-Haroush A, Bar J, Fisch B. C-reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum Reprod. 2004;19(2):357–9.
31. Wunder DM, Kretschmer R, Bersinger NA. Concentrations of leptin and Creactive protein in serum and follicular fluid during assisted reproductive cycles. Hum Reprod. 2005;20(5):1266–71.
32. Robinson S, Pemberton P, Laing I, Nardo LG. Low grade inflammation, as evidenced by basal high sensitivity CRP, is not correlated to outcome measures in IVF. J Assist Reprod Genet. 2008;25(8):383–8.