Medical Journal of Babylon
Vol. 12- No. 4:948 -955, 2015
http://www.medicaljb.com
ISSN 2312-6760©2015 University of Babylon

Original Research Article

Relation of Follicular Fluid C-Reactive Protein Level to Intracytoplasmic Sperm Injection Outcome at Al-Najaf Fertility Centre

Ban Dhahir Thabbah[1*] Sami Raheem Al-Katib[2] Basima Shamki Al-Ghazali[2]
[1] Ministry of Health Babylonian Health Center, Hilla Teaching Hospital , Hilla , IRAQ
[2] Faculty of Medicine , University of Kufa , Kufa , Najaf , IRAQ

[*]E-mail: ban_dahir@yahoo.com

Accepted 22 November , 2015

Abstract

C-reactive protein is a protein that act as a sensitive marker in inflammatory processes, rises following hormonal stimulation it may affect fertility and intracytoplasmic sperm injection (ICSI) outcome. this study aimed to measure follicular fluid proinflammatory mediator as C-reactive protein and their relation controlled ovarian hyperstimulation protocols and to interpret these findings according to the cause of subfertility and subfertility treatment outcome. This study was carried out on 110 subfertile women aged between 18-45year (30.01±6.02), referred to fertility clinic in Al-Sadder teaching hospital at AL-Najaf city who classified into four group according to the cause of subfertility, estradiol(E2) also measured on day of HCG and CRP were measured in follicular fluid collected on day of pickup using special ELISA kit then correlate the result to ICSI outcome. The Result of this study reported that the pregnancy rate was 34.55% for embryo transferred after 48-72 hours, there was non-significant increment in pregnant than non-pregnant women regarding FF-CRP (P>0.05), also non-significant difference in FF- CRP in subfertile women according to cause of subfertility(p=0.303), higher FF-CRP value were in ovulatory factor female. Regarding ICSI parameters, there was positive non-significant correlation between FF- CRP and embryo number, Grade I, Grade II, embryo transferred, fertilization rate. so conclusion of this study minimal increment in these inflammatory markers would aid and share in success ICSI outcome.

Key words: CRP, follicular fluid CRP, E2, intracytoplasmic sperm injection.

Abbreviations: E2: estradiol, COH: controlled ovarian hyperstimulation, PCOS: polycystic ovarian syndrome, NO: number, GI: grade I, GII: grade II.

الخلاصة

بروتين c التفاعلي هو البروتين الذي يعمل كعلامة تحسس لعملية الالتهاب الحاصلة في الجسم ويرتفع بعد التنشيط الهرموني وقد يؤثر على الخصوبة ونتائج الحقن المجهري.وتهدف هذه الدراسة الى قياس مستوى وسائط الالتهاب في السائل الحويصلي مثل بروتين c التفاعلي وعلاقته ببروتوكولات تنشيط المبيض ولترجمة هذه النتائج حسب سبب العقم ونتائج علاج العقم.طبقت هذه الدراسة على ١١٠ امرأة عقيمة تتراوح اعمارهن بين ١٨ –٤٥ سنة بمتوسط (٣٠.٠١±٦.٠٢) وقد صنفوا إلى اربع مجاميع حسب سبب العقم. وقد تمت الدراسة في مركز الخصوبة في مستشفى الصدر التعليمي في مدينة النجف للفترة من اذار الى تشرين الاول 2014 .وتم قياس مستوى الاستروجين في الدم في يوم اعطاء هرمون.HCG وفي يوم التقاط البويضة يتم قياس بروتين cالتفاعلي في السائل الحويصلي باستخدام جهاز الإيلايزا ومقارنة النتائج بنتائج الحقن المجهري.وقد سجلت هذه الدراسة ان معدل الحمل لدى النساء بصورة عامة كان ٣٤.٥٥%للأجنة المسترجعة بعد ٤٨–٣٦ ساعة وكذلك فإن هناك زيادة غير معنوية بالنسبة لبروتين cالتفاعلي في السائل الحويصلي لدى النساء الحوامل عنه في غير الحوامل. كذلك بالنسبة لمستواه بين النساء حسب سبب العقم فإن هناك زيادة غير معنويه بين النساء ذوات العقم المتعلق بالتبويض. اما فيما يخص نتائج الحقن المجهري فإن هناك علاقة ايجابية غير معنوية بين مستوى بروتين cالتفاعلي وعدد الاجنة ,الاجنة من الدرجة الاولى والثانية ,الاجنة المنقولة ومعدل الاخصاب. وعليه فإن هذه الدراسة استنتجت ان الزيادة البسيطة في وسائط الالتهاب ستساعد على نجاح عملية الحقن المجهري.

الكلمات المفتاحية: بروتين cالتفاعلي ,الاستروجين, بروتين cالتفاعلي في السائل الحويصلي.

Introduction

C-reactive protein (CRP) can be defined as an acute phase protein and its production is from the liver. Researcher found that after estrogen administration CRP was increased [1]. The upsurge in the concentration of IL-6 that secreted from macrophage will results in acute phase response [2]. Other site of IL-6 secretion is adipocytes [3], also is secreted in response to many acute and chronic inflammatory situation and act a primary defense such as viral, bacterial, or fungal infections, malignancy, rheumatic and other inflammatory diseases, necrosis and tissue injury. Leading to release of interleukin-6 and other cytokines, which in turn promoting the liver to synthesize CRP and fibrinogen [4].

Also in other studies researchers report that follicular fluid CRP level was lower than serum CRP indicating that the ovarian production of CRP was little or absent, and previous literatures lack a data about granulosa cell CRP production. Challis et al, stated that tissue remodeling and effective implantation require inflammatory microenvironment (that accompanied by increased chemokines, cytokines and their receptors) in the first trimester of pregnancy [5]. On other hand too much inflammatory reaction may lead to recurrent abortion, further pregnancy complications like preterm labour or pre-eclampsia [6,7].

Materials and Methods

The protocol of our study was planned according to Kufa declaration for the ethical philosophies of medical research, as a prospective cohort study with acceptation from the committee of ethical research at Physiology and Obstetrics and Gynecology Departments, Faculty of Medicine, University of Kufa. A total of 120 included subfertile couples referred to the fertility center of Al-Sadder teaching hospital in Al-Najaf city between the period from March to October 2014. All women considered, and underwent 120 IVF-ICSI cycles after a certain ovarian stimulation protocol, 110 reached the ovum pick up stage, the remained either cancelled cycle or had no stimulated oocyte by ultrasound. All 110 were distributed into 4 subgroups in relation to cause of subfertility: 32 female factor (Ovulatory factor, endomatriosis), 54 male factor, 16 Tubal and 8 Unexplained subfertility. all were candidate for ICSI according to the indication. they were of reproductive age ranging from 18-45 years old. Free from hepatitis and HIV (by screening test). Women with a recent history of infection or inflammation or surgery or any condition that may suspected to increase CRP were excluded from the study. all of these women underwent ovulation induction with different hyperstimulation protocol: either long (No=14), short (No= 66) and antagonist (No= 30) protocols depending on hormonal conditions, timing, and ovarian reserve status of the women on the discretion of the clinician. On day of HCG prescription, Serum sample obtained from women to measure E2 by Biomerieux, SA, France kit using VIDAS, After a period of time under vaginal ultrasound guidance HCG injection was given to trigger the final stages of oocyte maturation with oocyte pick up guided by ultrasound was performed 34-36 h later and follicular fluid was aspirated from patient on day of pickup and centrifuged and freezed till the time of measurement using C-Reactive Protein (CRP) ELISA Kit (Product No. ABIN1874314). Embryos classified according to their morphology and percentage of fragmentation, then good quality embryos were returned after a period ranging from 3-5 days.

Then after 2weeks HCG was measured in blood to determine biochemical pregnancy.

Statistical analysis in this study was performed using SPSS(Statistical Package for Social Science; Version 20)program. Expression of values as mean ± SD, independent t-test was used. Also one-way ANOVA was used to evaluate differences of means among multiple groups. Pearson's correlation analysis had been used to correlate among the measured variables and other clinical variables or data.

To investigate the expectedness of ICSI variables and E2, FF-CRP levels for pregnancy we use Receiver operating characteristic (ROC) curves,. Also by this analysis we can determine the sensitivity and specificity and the result were described as mean ±SD., with p value less than 0.05 that was reflect a statistical significant[8].

Results

Pregnancy rate was 34.55% (38 pregnant, 72 non pregnant).

Table 1:Mean and standard deviation (SD) of FF-CRP, serum E2 in studied group and between pregnant and non-pregnant women

TEST	All patients (mean ±SD)	Pregnant (mean ±SD)	Non pregnant	P value
Follicular CRP mg/l	0.24±0.76mg/l	0.46±1.17	0.13±0.4	0.068
Peak E2 (Pmol/L)	2065±909.5	2242.8±910.2	1937± 905.8	0.282

One of the inflammatory markers in the follicular fluid is the CRP. One way ANOVA test describes the distribution of FF CRP according to the cause of subfertility figure (1) p=0.303, higher follicular fluid value were in ovulatory factor female this may be attributed to inflammatory a etiology of ovulatory factor which was mainly due to PCOS.

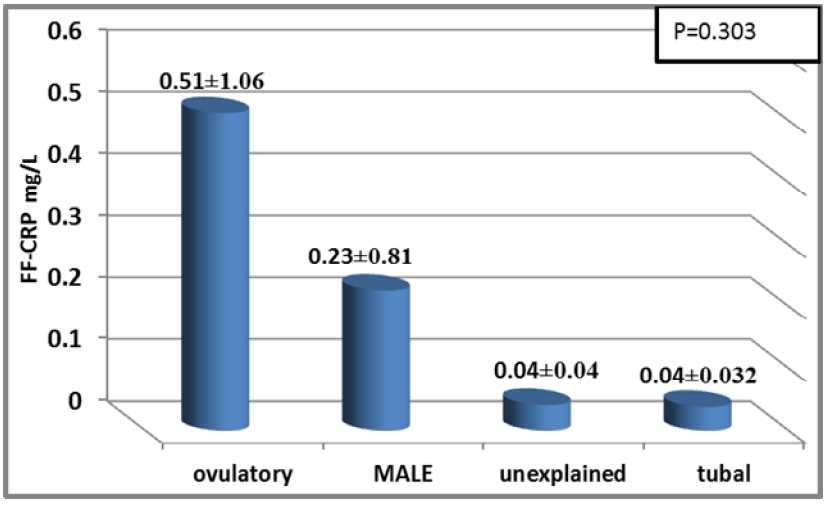

Figure 1:Distribution of FF- CRP according to the cause of subfertility

C- reactive protein between different used stimulation protocols

there was non-significant difference in FF CRP between different protocol used (p value >0.05), p=0.196.

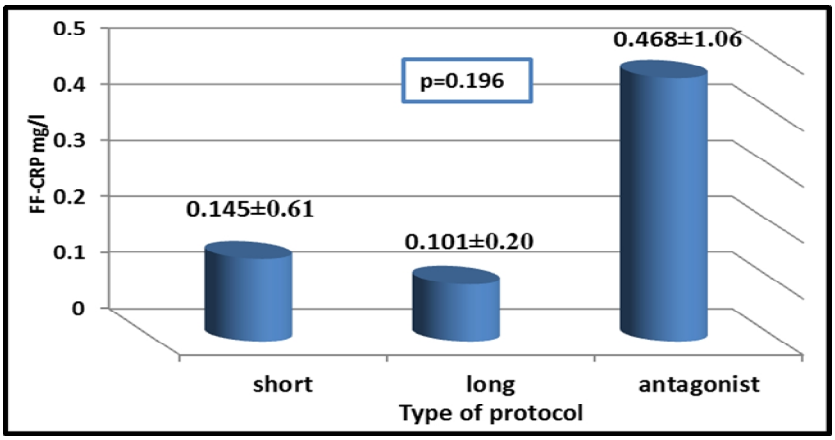

Figure 2: mean and standard deviation in CRP among patient according to the used protocol.

There were significant or highly significant correlation between peak E2 and most ICSI outcome (oocyte number, MII, injected, pronuclear) with significant correlation with embryo number and nearly significant positive correlation with GI,GII and increase pregnancy rate, with positive significant correlation between FF-CRP and PN, and positive non significant correlation with most ICSI variables as shown in table (2):

Table 2: Correlation of peak E2and FF-CRP to some ICSI variables.

ICSI outcome variable	Peak E2		FF- CRP	
	r	p	r	p
Oocyte number	0.594**	0.0001	0.195	0.080
MII	0.619**	0.0001	0.192	0.086
injected	0.603**	0.0001	0.210	0.060
pronuclear	0.579**	0.0001	0.248	**0.028***
Embryo NO.	0.389*	0.012	0.183	0.107
GI	0.287	0.076	0.164	0.159
GII	0.292	0.071	0.049	0.615
Embryo transferred	0.304	0.056	0.117	0.302
FR%	-0.114	0.503	0.204	0.086
Pregnancy	0.168	0.282	0.153	0.171

MII: metaphase II, GI,II: grade I,II embryo, FR: fertilization rate *level of significance below 0.05, ** level of significance below 0.001. r: correlation coefficient, p: level of significance.

Regarding the correlation between peak E2 and FF-CRP there was positive non significant correlation between E2 & CRP

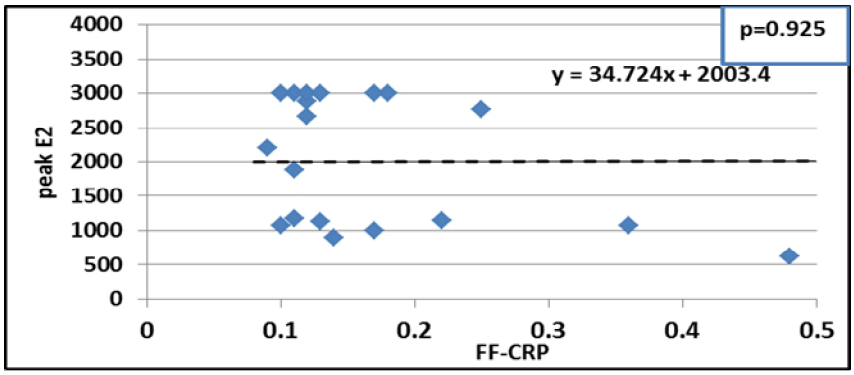

Figure 3: Correlation between peak E2 and follicular fluid CRP

ROC analysis revealed that the CRP had the largest area under the curve (AUC:0.634 indicating that threshold of 0.45 gave a sensitivity of 82%, and specificity of 42%). Followed by E2 (AUC) 0.602, a threshold of 947.5 gave sensitivity of 100% and specificity 24%.

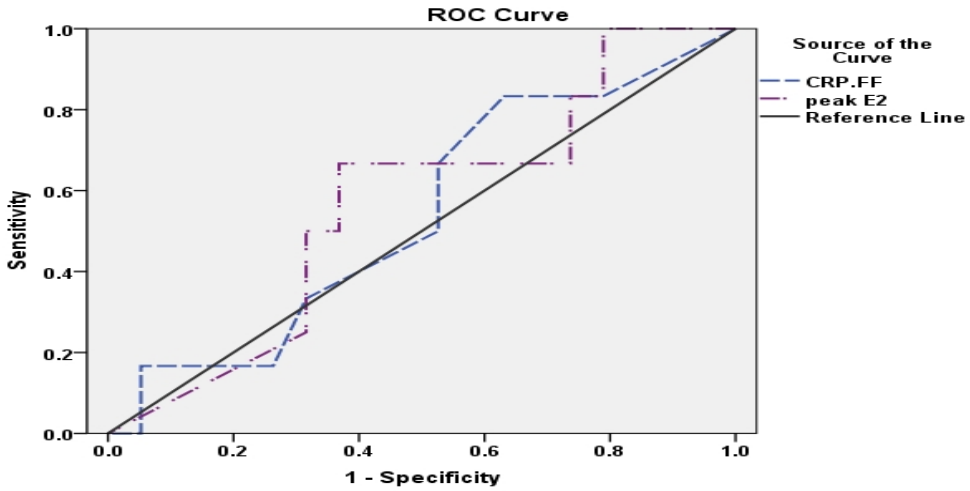

Figure 4: Estimated area under curve (AUC) and predictive cut-off point values for the individual some seroimmunological and hormonal parameters with the receiver operating characteristics (ROC) curve analysis between pregnant and non-pregnant women.

variables	AUC	Cut off point	sensitivity	specificity	CI 95%	
FF -CRP	0.634	0.45	82%	42%	0.392	0.655
Peak E2	0.602	947.5	100%	24%	0.429	0.575

CI: confidence interval

Discussion

Pregnant women having higher peak E2 than non-pregnant one with p value >0.05 as in table (1) that was in agreement with Al-Ghazali & Al-Jarrah who prove that E2 level at day of HCG injection was more in women that have got pregnancy than in women that haven't got pregnancy which reflects good ovarian response, but there were no significant differences [9]. The follicular fluid CRP mean was 0.24±0.76mg/l, while its level in pregnant women was 0.46±1.17and in non-pregnant women was 0.13±0.4 with p value 0.068 as shown in table (1) these result was in agreement with Orvieto *et al.*, they found that on day of pick up FF-

CRP levels was significantly increased beyond the cutoff point of 0.5 mg/dl, meaning a presence of an inflammatory reaction. Orvieto et al. found that although the positive correlation was found on HCG day, there was no correlation between CRP and E2 levels on day of pickup because CRP still increased although E2 decreased meaning that the inflammatory response was significantly stimulated by the exogenous HCG [10]. Furthermore the above result was in agreement with Sacks, et al who found the presence of significant high CRP levels in pregnant women than non-pregnant beyond IVF/ICSI treatment (COH) protocol especially 2 weeks after oocyte retrieval and collection [11].

During controlled ovarian hyper-stimulation (COH), serum CRP was significantly elevated until the peak estradiol concentration, and following HCG administration still there was further increment. With no observed significant variation between follicular fluid and serum and CRP levels. also no correlation existed between serum CRP level and subfertility cause, variables of ICSI treatment or pregnancy rate [10, 25]. CRP does not have diurnal alterations, but administration of exogenous estrogen increases its level [12].

Redman and Sargent reported that the presence of inflammatory reaction in pregnant women rather than in non-pregnant, consolidating in their result and confirming that ordinary pregnancy considered as inflammatory condition as previously mentioned [13]. Further studies reporting by endometrial biopsy that endometrial local injury resulted in elevated implantation and pregnancy rates in a cycle preceding IVF/ICSI treatment [14- 16], that might be explained possibly as a local injury stimulated decasualization and the wound healing effect, secretion of growth factors and inflammatory mediators, and accompanied by invasion of immune cells.

One way ANOVA test describes the distribution of FF-CRP according to the cause of subfertility figure (1), higher follicular fluid value were in ovulatory factor female this might be attributed to inflammatory a etiology of ovulatory factor which was mainly due to PCOS, with non-significant difference between group of subfertility (p=0.303). this result was proved by other researcher , Several investigations had proposed that women with chronic anovulation and polycystic ovary syndrome (PCOS) were in a constant chronic inflammatory status, while others describe chronic anovulation and PCOS as a low-grade inflammation disorder [17-19].

In PCOS women positive correlation was existed between CRP levels and insulin resistance, fatty mass and body weight with 2 fold elevation in circulating CRP [19- 21].

While in figure (2) there was non-significant difference in FF-CRP between different protocol used (p value >0.05), p=0.196.this result was consolidated by Arefi, *et al*., 2010[22] reported that there was different patterns(that may be considered as indicator of ICSI treatment successful outcomes) of increment in CRP level as a result of inflammatory process caused by ovulation induction. Especially on embryo transfer day relative small increment in CRP level accompanied successful outcome .

Regarding effect of CRP on embryo quality and fertilization rate (Table 2) there was positive non-significant correlation between FF-CRP and most ICSI variables. These result were in agreement with Bódis, József, *et al*., 2014 [23] whom found that CRP in serum and FF did not influence the number of oocytes and embryos.

Sacks, et al demonstrating that any difference in maternal-fetal cross point might be associated with abnormal CRP response whether deficient or elevated that may occur in women with abortion. Beyond these results CRP might be considered as a predictor marker or screening method for selection of women who need immunotherapy even in non

ICSI conception and measuring CRP concentration in pregnancy failure [11].

Jabbour *et al.* pointed out the importance of inflammatory pathways throughout reproductive physiology, from ovulation, to menstruation, implantation and onset of labor, all now gradually perceived as inflammatory processes [24]. Here presented study suggests that creation of good embryo quality now also might had to be added as a process dependent on inflammatory pathways.

Albertini recently described the ovaries as immunological "hot-spots" [25] based on the recognition that increasing numbers of fertility-associated genes in the ovary also have immunological functions.

References

1. Kluft C, Leuven JA, Helmerhorst FM and Krans HM (2002) Pro inflammatory effects of oestrogens during use of oral contraceptives and hormone replacement treatment. Vascul Pharmacol 39,149–154.
2. Pepys MB and Hirschfield GM. (2003): C-reactive protein: a critical update. The Journal of Clinical Investigation; 111(12): 1805–12.
3. Lau DC, Dhillon B, Yan H, Szmitko PE and Verma S. (2005): Adipokines: molecular links between obesity and atheroslcerosis. American Journal of Physiology. Heart and Circulatory Physiology; 288(5): H2031–41.
4. Aantovani A, Garlanda C, Doni A and Bottazzi B. (2008): Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. Journal of Clinical Immunology; 28 (1): 1–13.
5. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF and Petraglia F. (2009): Inflammation and pregnancy. Reprod Sci; 2: 206-15.
6. Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O and Martal J. (2004): TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol; 2: 93-119.
7. Calleja-Agius J, Jauniaux E, Pizzey AR and S. Muttukrishna. (2012): Investigation of systemic inflammatory response in first trimester pregnancy failure.
8. Daniel WW (1999): 7th ed. New York: John Wiley & Sons; Biostatistics: a foundation for analysis in the health sciences.
9. Al-Ghazali, B. S. and Al-Jarrah D.M. (2013): Factors Affecting Intra-Cytoplasm Sperm Injection (ICSI) and Pregnancy Outcome in the Fertility Center of Al –Najaf City. The Iraqi Postgraduate Medical Journal vol.12, supplement.
10. Orvieto R, Chen R, Ashkenazi J, Ben-Harush A, Bar J and Fisch B (2004a): C-reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum Reprod; 19:357–9.
11. Sacks GP, Seyani L, Lavery S and Trew G. (2004): Maternal C-reactive protein levels are raised at 4 weeks gestation. Hum Reprod; 19: 1025–30 .
12. de Maat MP, Madsen JS, Langdahl B, Bladbjerg EM, Tofteng CL, Abrahamsen B, et al. 2007:Genetic variation in estrogen receptor, C-reactive protein and fibrinogen does not predict the plasma levels of inflammation markers after long term hormone replacement therapy. Thromb Haemost; 97: 234-239.
13. Redman CW and Sargent IL.(2010): Immunology of pre-eclampsia. Am J Reprod Immunol; 6: 534-43.
14. Barash A[1], Dekel N, Fieldust S, Segal I, Schechtman E, Granot I.(2003 Jun): Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. Fertil Steril.; 79 (6): 1317-22.
15. Zhou L, Li R, Wang R, Huang HX and Zhong K. (2008): Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. Fertil. Steril; 89:1166–76.

16. Karimzadeh M.A. , Ayazi R.M. and Tabibnejad N. (2009): Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing in vitro fertilisation/intra cytoplasmic sperm injection: a randomized clinical trial Aust. N. Z. J. Obstet. Gynaecol., 49, pp. 677–680.
17. Dhindsa G, Bhatia R, Dhindsa M and Bhatia V. (2004): Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome. J Postgrad Med.; 50(2):140-44.
18. Kousta E, Tolis G and Franks S. (2005): Polycystic ovary syndrome. Revised diagnostic criteria and long-term health consequences. Hormones (Athens); 4(3): 133-47.
19. Repaci A, Gambineri A and Pasquali R. (2011): The role of low-grade inflammation in the polycystic ovary syndrome. Mol Cell Endocrinol.;335(1): 30-41.
20. Benson S, Janssen OE and Hahn S. et al. (2008): Obesity, depression, and chronic low-grade inflammation in women with polycystic ovary syndrome. Brain Behav Immun; 22(2):177-84.
21. Escobar-Morreale HF, Luque-Ramirez M and Gonzalez F.(2011): Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. Fertil Steril.; 95:1048–58. e1041–1042.
22. Arefi S., Babashamsi M., Panahi, P.S., Saruiy L.A. and Zeraati H. (Autumn 2010): C-reactive protein level and pregnancy rate in patients undergoing IVF/ICSI. Iran J Reproductive Med Vol.8. No.4. pp: 197-202.
23. Bódis József , Peti AM. , Sulyok Endre , Kovács L., Gábor and Várnagy Ákos (2014): Serum and follicular fluid fetuin-A in women undergoing *in vitro* fertilization. Clin Chem Lab Med, 59 (9). pp. 1313-1318.
24. Jabbour HN, Sales KJ, Catalano RD and Norman JE. (2009): Inflammatory pathways in female reproductive health and disease. Reproduction; 138: 903-19.
25. Albertini DF. (2012): Searching for answers to the riddle of ovarian aging. J Assist Reprod Genet; 29:577-8.
26. Orvieto R, Schwartz A, Bar-Hava I, Abir R, Ashkenazi J, La-Marca A and Ben-Rafael Z. (2000): Controlled ovarian hyperstimulation - a state of endothelial activation. Am J Reprod Immunol; 44: 257–60.