Soluble CD30 in Normal Pregnancy Pre-Eclampsia and Recurrent Pregnancy Loss

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ABSTRACT

Background: Normal pregnancy is thought to be dependent on Th2 deviation, while Recurrent Pregnancy Loss (RPL) and Pre-eclampsia (PE) appear to be biased toward the Th1 immune response. It is believed that the soluble form of CD30 (sCD30) is an index of Th2 immune response or modulator of Th1/Th2 responses. **Objective:** The aim of this study was determination of the sCD30 level in RPL and PE patients. **Methods:** The sCD30 level was measured in sera of a group of normal non-pregnant women (N=43) and compared with normal pregnancy at the first (N=42) and third (N=42) trimester. Furthermore, the level of sCD30 in the normal first and third trimester pregnancies were compared with that of RPL (N=38) and severe pre-eclamptic (N=41) patients, respectively. sCD30 levels were measured by ELISA method and student *t-test* was used for statistical analysis. **Results:** The mean level of sCD30 at the first trimester in normal pregnancy was significantly elevated as compared with normal non-pregnant women (21.4 vs. 15.2 ng/ml, $p<0.0001$). A significant difference between sCD30 concentration at the first and third trimester of normal pregnancies was also observed (21.4 vs. 14.3 ng/ml, $p<0.0001$). Interestingly, the sCD30 concentration did not show any significant changes at the first trimester of normal pregnancy as compared with RPL (21.4 vs. 20.9 ng/ml) and third trimester of normal pregnancy as compared with PE (14.3 vs. 13.1 ng/ml). **Conclusion:** The data of this study indicated that the concentration of sCD30 in serum during pregnancy period is not associated with RPL or PE and serum sCD30 is not a good correlate of Th2 immune responses in pregnancy.

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INTRODUCTION

Recurrent pregnancy loss (RPL) and pre-eclampsia (PE) are the most common complications of pregnancy and leading causes of major maternal and fetal morbidity and mortality of human pregnancies. RPL refers to at least two previous pregnancy losses prior to the 20th week of gestation (1). While in approximately 60% of RPL cases the etiological factors are known, in the remaining 40% of the cases, the etiology of abortion is unknown and the condition is classified as unexplained RPL (URPL) (2).

Pre-eclampsia (PE) is characterized with new onset of maternal hypertension with sustained systolic blood pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg and proteinuria greater than 300 mg per 24 hours (3). Pre-eclampsia is considered mild if hypertension and proteinuria as previously defined are present but not in the severe range (4). Severe pre-eclampsia is defined with the presence of at least greater than 160 mmHg systolic or 110 mmHg diastolic blood pressure along with at least 5 gr of protein in a 24 hour urine collection or 3+ urine dipstick testing. Several factors including immune reaction disorders are thought to be associated with RPL or pre-eclampsia (5,6).

Cytokines are important immune factors that regulate immune responses. During pregnancy period, cytokines are produced by both trophoblast and maternal immune cells and play a major role in communication between fetus and mother as well as homeostasis of immune responses (7). Based on the pattern of produced cytokines, T helper (Th) cells are divided into two major types. Th1 cells produce a group of cytokines including IFN-γ and IL-2 which are involved in cellular immunity while, Th2 cells are involved in humoral immunity through production of cytokines such as IL-4, IL-5 and IL-13 (8).

In the pregnancy period cytokine imbalance is thought to be an important mechanism for pregnancy related disorders. Normal pregnancy is thought to be a deviation of the immune system towards Th2 type of immunity, while most pregnancy related complications are believed to be a bias toward Th1 immune response (9). It is believed that both RPL and pre-eclampsia are characterized by exaggerated Th1 responses. Several studies have confirmed higher production of Th1 cytokines in RPL and pre-eclamptic pateints, as compared with normal pregnant women (10,11).

CD30 is a 120 KD surface glycoprotein molecule which belongs to the tumor necrosis factor receptor superfamily (12). While CD30 is expressed by activated Th2 cells as a surface molecule, the soluble form of the CD30 (sCD30) is also produced by proteolytic cleavage of CD30 and secretion into the plasma (13). sCD30 is a 88 KD molecule and is consider as an index of Th2 cell activation and modulator of Th1/Th2 responses (14,15). It has been reported that during a normal pregnancy serum concentrations of sCD30 is elevated (16). This observation is in line with the Th2 hypothesis in normal pregnancy. After the important role of Th1/Th2 cytokine balance in pregnancy outcome was revealed, a few studies have been done to investigate the relationship between maternal serum sCD30 concentration and pregnancy related disorders, including fetal intrauterine growth restriction, small for gestational age pregnancies and pregnancies complicated with acute pyelonephritis (16-18). Interestingly, scarce conflicting results regarding the correlation between maternal serum sCD30 level and pregnancy outcome in RPL and pre-eclamptic patients have been reported.

While Kusanovic and coworkers reported a significant lower concentration of maternal sCD30 in the sera of pre-eclamptic women (16), their results were not confirmed in a

recent publication by Laskowska and coworkers (19). Moreover, till Aug 2012, there is only one published article in PubMed, regarding the relationship between sCD30 and recurrent abortion. In this report, Makhseed and coworkers have shown that the maternal serum concentration of sCD30 is not associated with pregnancy outcome in recurrent spontaneous aborters (20). Considering scarce data regarding the prognostic value of the sCD30 level in RPL or pre-eclamptic patients, the present study was undertaken in a group of Iranian women to evaluate the sCD30 concentrations in RPL and PE.

MATERIALS AND METHODS

Subjects. In total, 206 ethnicity matched women after obtaining the informed consent, participated in this study, approved by local ethics committee, Shiraz University of Medical Sciences, Shiraz, Iran. All participants were diagnosed and investigated clinically or serologically, if needed, by the same gynecologist. Our subjects were divided to five following groups:

- Group A: Forty three normal non-pregnant women without any history of pregnancy between 21-33 years old.
- Group B: Forty two normal pregnant women at first trimester (gestational week between 8-12), with history of at least two previous pregnancies and without history of abortion formed our normal first trimester group. Group B women were age and ethnic match with group A.
- Group C: Forty two normal primiparous pregnant women (gestational week between 32-35) as normal third trimester normal group.
- Group D: Thirty eight primary URPL women with at least two sequential abortions with the same partner. Group D patients were age and gestational week match with group B. URPL cases were diagnosed by a gynecologist based on clinical data and laboratory results. To select URPL patients and exclude RPL ones from this study, all patients were evaluated for normal anatomical, hormonal and chromosomal criteria and the absence of infections (toxoplasma, cytomegalovirus, rubella, HIV, Chlamydia, hepatitis B and C), anti-thyroid antibody and anti-phospholipid antibodies (including lupus anticoagulant, anti-cardiolipin and β2-glycoprotein antibodies).
- Group E: Forty one primiparous pregnant severe pre-eclamptic women. Group E patients were age and gestational week match with group C. The diagnosis of severe PE was based on the new blood pressure, 160 mmHg or higher systolic or 110 mmHg or higher diastolic on two occasions at least 6 hours apart, and plus onset of proteinuria, 5 gr or more of protein in a 24 hours urine collection or 3+ or greater on urine dipstick testing of two random urine samples collected at least 4 hours apart. Demographic characteristics for all participated women are summarized in the Table 1.

Sampling and the Measurement of sCD30 Level. Two ml of peripheral blood from all participants were collected. Blood samples were taken from non-pregnant group A women in the luteal phase.

Sera were separated from blood after centrifuging at 1000 x g for 10 minutes. All sera were stored at -70°C until assay. sCD30 levels were measured by ELISA method (BenderMed Systems, Vienna, Austria). Assay range of the ELISA kit was between 0.33-100 ng/ml. Intra- and Inter-assay coefficients of variation for measurement of sCD30 were 5.1 and 4.3 percent, respectively.

Statistical Analysis. Statistical analysis were carried out using (SPSS), version 16 for Windows (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was first confirmed by using the Kolmogorov-Smirnov test, Student's *t-test* was used for statistical analysis and P-values less than 0.05 were considered as significant.

RESULTS

A significant difference between sCD30 concentration in normal non-pregnant as compared to normal pregnant women at the first and third trimester was observed (Table 1 and Figure 1). The mean level of sCD30 in Group A (normal non-pregnant women) was 15.2 ng/ml, while the mean level of sCD30 in Group B (normal first trimester pregnant women) was 21.4 ng/ml ($p<0.0001$). A significant difference between sCD30 concentration at the first and third trimester of a normal pregnancy (group B vs. group C) was also observed (21.4 vs. 14.3 ng/ml, $p<0.0001$, Table 1 and Figure 1).

Table 1. Demographic Characteristics and sCD30 level of cases and controls.

Parameter	Group A (n=43)	Group B (n=42)	Group C (n=42)	Group D (n=38)	Group E (n=41)	P Value
Maternal age (years)	26.4 ± 3.2	27.1 ± 3.3	23.5 ± 4.1	28.2 ± 3.4	25.2 ± 3.1	NS
Gestational week	-	11 ± 1	34 ±1	12 ± 1	33 ± 2	NS
Systolic blood pressure (mmHg)	109 ± 11	105 ± 7	111 ± 14	121 ± 13	155 ± 14	*
Diastolic blood pressure (mmHg)	73 ± 5	71 ± 8	75 ± 9	71 ± 8	98 ± 24	**
Maternal sCD30 level (ng/ml)	15.2 ± 3.3	21.4 ± 3.9	14.3 ± 3.4	20.9 ± 5.3	13.1 ± 3.2	***

Group definitions: Group A= Non-pregnant, Group B= Normal pregnant at first trimester, Group C= Normal pregnant at third trimester, Group D= URPL pregnant, Group E= Severe pre-eclamptic pregnant women. All values are given as mean ± SD.
* P<0.0001 for Group E versus all other groups
** P<0.0001 for Group E versus all other groups
*** P<0.0001 for maternal sCD30 level Group A versus Group B and Group B versus Group C

As indicated in Table 1 and Figure 1, the sCD30 concentration did not show any significant changes at the first trimester of normal pregnancy (group B) when compared with URPL (group D) subjects. The mean concentrations of sCD30 in group D and B calculated to be 20.9 ng/ml, and 21.4 ng/ml, respectively. The same result was observed when the mean level of sCD30 in group C (normal third trimester pregnant women) was compared with that of severe pre-eclamptic patients (group E) (14.3 vs. 13.1 ng/ml, Table 1 and Figure 1).

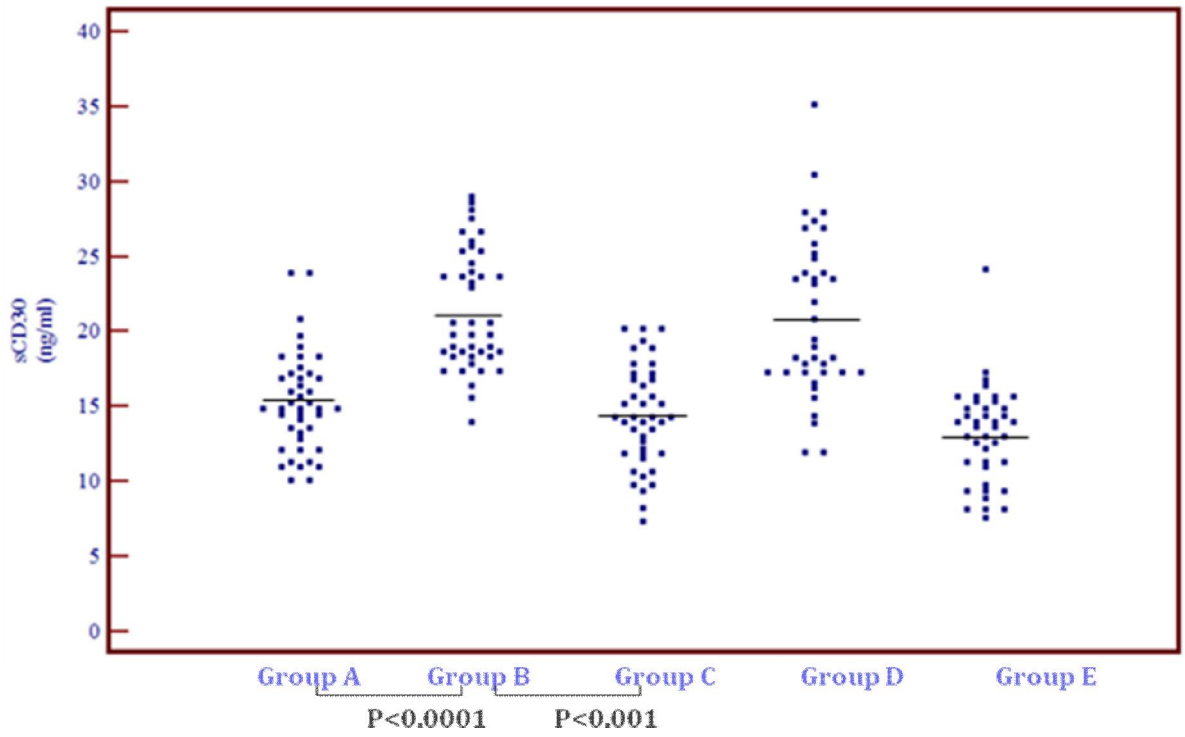

Figure 1. Dot blot graph of sCD30 concentration in different groups indicating the mean level for data presented in Table 1. Group definitions: Group A= Non-pregnant, Group B= Normal pregnant at first trimester, Group C= Normal pregnant at third trimester, Group D= URPL pregnant, Group E= Severe pre-eclamptic pregnant women.

DISCUSSION

It is widely believed that in the first trimester of a normal pregnancy a Th2 shift dominates the immune response. While in the third trimester, a Th1 shift in immune responses to the fetus occurs. Therefore, in this study we investigated the serum concentration of sCD30, as a correlate of Th2 responses, in URPL or PE cases and compared with sCD30 levels in normal pregnant and non-pregnant women to find an association between sCD30 level and RPL or PE. The results can provide information to be used in future evaluation of prognostic or predictive value of sCD30 level in pregnancy related disorders. Our results indicated that sCD30 concentration significantly increased with the onset of pregnancy in the first trimester of a normal pregnancy and then decreased in the third trimester (Table 1, Figure 1). In line with our results, several previous studies indicated a Th1/Th2 bias in first and third trimester of a normal pregnancy (21-23). On the other hand our results confirms the previous reports regarding elevation of sCD30 following normal pregnancy (16). However, our results regarding the sCD30 level in pre-eclamptic or recurrent aborters did not match our hypothesis. In the only one published data regarding the prognostic value of sCD30 level in recurrent abortion, Makhseed and coworkers did not detect any significant correlation between sCD30 level and RPL which is in line with the results of the present study (20). On the other hand while the report from Kusanovic and coworkers indicated a significant lower concentration of maternal sCD30 in the sera of pre-eclamptic women (16), the results of our study is in line with the results of another recent published work

by Laskowska and coworkers that did not observe any significant change in sCD30 level in pre-eclamptic women (19). The traditional viewpoint is that Th0 immune system is skewed towards a Th2 type of immune system by the fetus (24). This type of immune system is in favor of preserving the fetus against the immune system of mother. Regarding the effect of Th1/Th2 balance in pregnancy outcome, several studies have shown that Th1 type cytokines are increased while Th2 ones are decreased in pre-ecplamptic or recurrent aborter women (10,11,24,25-28). In contrast, there are several studies indicating a Th2-dominant immunity in recurrent abortion or pre-eclampsia (29,30). Therefore, it seems that the Th1/Th2 paradigm is now insufficient to explain the mechanism of tolerance of maternal immune cells to the fetus or some pieces of the puzzle are not yet found. Indeed, the Th1/Th2 paradigm has been expanded into the Th1/Th2/Th17 and regulatory T (Treg) cell paradigm (31). Th17 cells are a subtype of T lymphocytes that secrete IL-17 and act in favor of inflammation and graft rejection in the immune system, a role similar to that of Th1 cells (31-34). Interestingly, several studies have shown a higher activity of Th17 cells in abortions or pre-clampsia (35,36). Tregs, on the other hand, reduce the proliferation of immune cells and induce tolerance in the immune system and, therefore, act in favor of fetus preservation against maternal immune system (37). Another important point is that cytokine milieu and immune cell composition of deciduas is the primary mediators of maternal-fetal immunity. Consequently, blood cells and cytokines can be different from that of deciduas. This can lead to problems regarding measurement of blood factors, which can give us false values that are not associated with those of the deciduas. In conclusion the data of this study indicated that the level of sCD30 in serum during pregnancy period is not associated with RPL or PE. Finally it may be concluded that the measurement of sCD30 in serum is not a good correlate of Th2 immune responses in pregnancy.

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REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Difinitions of infertility and recurrent pregnancy loss. Fertil Steril. 2008; 90:S60.
2. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril. 1996; 66:24-9.
3. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011; 7: 467-74.
4. Turner JA. Severe Preeclampsia: Anesthetic Implications of the Disease and its Management. Am J Ther. 2009; 16:284-8.
5. Pandey MK, Rani R, Agrawel S. An update in recurrent spontaneous abortion. Arch Gynecol Obstet. 2005; 271:95-108.
6. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in preeclampsia. Mol Aspects Med. 2007; 28:192-209.
7. van Mourik MS, Macklon NS, Heijnen CJ. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol. 2009; 85:4-19.
8. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986; 136:2348-57.
9. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today. 1997; 18:478-82.
10. Saini V, Arora S, Yadav A, Bhattacharjee J. Cytokine in recurrent pregnancy loss. Clin Chim Acta. 2011; 412:702-8.
11. Saito S, Sakai M. Th1/Th2 balance in preeclampsia. J Reprod Immunol. 2003; 59:161-73.
12. Smith CA, Gruss HJ, Davis T, Anderson D, Farrah T, Baker E, et al. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell. 1993; 73:1349-60.

13. Hansen HP, Kisseleva T, Kobarg J, Horn-Lohrens O, Havsteen B, Lemke H. A zinc metalloproteinase is responsible for the release of CD30 on human tumor cell lines. Int J Cancer. 1995; 63:750-6.
14. Romagnani S, Del Prere G, Maggi E, Chilosi M, Caligaris-Cappio F, Pizzolo G. CD30 and type 2 T helper (Th2) responses. J Leukoc Biol. 1995; 57:726-30.
15. Hoshimoto K, Ohta N, Ohkura T, Inaba N. Changes in plasma soluble CD26 and CD30 during pregnancy:markers of Th1/Th2 balance? Gynecol Obstet Invest. 2000; 50:260-3.
16. Kusanovic JP, Romero R, Hassan SS, Gotsch F, Edwin S, Chaiworapongsa T, et al. Maternal serum soluble CD30 is increased in normal pregnancy, but decreased in preeclampsia and small for gestational age pregnancies. J Matern Fetal Neonatal Med. 2007; 20:867-78.
17. Laskowska M, Laskowska K, Oleszczuk J. Soluble CD30 in normotensive pregnant women with isolated fetal intrauterine growth restriction: a comparison with preeclamptic women. J Reprod Immunol. 2010; 86:122-5.
18. Kusanovic JP, Romero R, Esoinoza J, Gotsch F, Edwin S, Chaiworapongsa T, et al. Maternal serum soluble CD30 is increased in pregnancies complicated with acute pyelonephritis. J Matern Fetal Neonatal Med. 2007; 20:803-11.
19. Laskowska M, Laskowska K, Oleszczuk J. Evaluation of sCD30 level in maternal serum in pregnancies complicated by severe preeclampsia. Med Wieku Rozwoj. 2011; 15:73-8.
20. Makhseed M, Raghupathy R, Azizieh F, Farhat R, Hassan N, Bandar A. Circulating cytokines and CD30 in normal human pregnancy and recurrent spontaneous abortions. Hum Reprod. 2000; 15: 2011-7.
21. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol. 1996; 106:127-33.
22. Saito S, Umekage H, Sakamoto Y, Sakai M, Tanabe K, Sasaki Y, et al. Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with preeclampsia. Am J Reprod Immunol. 1999; 41:297-306.
23. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod. 2000; 15:713-8.
24. Saito S, Sakai M, Sasaki Y, Tanebe K, Tsuda H, Michimata T. Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin Exp Immunol. 1999; 117:550-5.
25. Makhseed M, Raghupathy R, Azizieh F, Omu A, Al-Shamali E, Ashkanani L. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. Hum Reprod. 2001; 16:2219-26.
26. Szarka A, Rigó J Jr, Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol. 2010; 11:59.
27. Molvarec A, Szarka A, Walentin S, Beko G, Karádi I, Prohászka Z, et al. Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia. Reprod Biol Endocrinol. 2011; 9:124.
28. Molvarec A, Shiozaki A, Ito M, Toldi G, Stenczer B, Szarka A, et al. Increased prevalence of peripheral blood granulysin-producing cytotoxic T lymphocytes in preeclampsia. J Reprod Immunol. 2011; 91:56-63.
29. Chaouat G, Lédée-Bataille N, Zourbas S, Ostojic S, Dubanchet S, Martal J, et al. Cytokines, implantation and early abortion: re-examining the Th1/Th2 paradigm leads to question the single pathway, single therapy concept. Am J Reprod Immunol. 2003; 50:177-86.
30. Peck A, Mellins ED. Plasticity of T-cell phenotype and function: the T helper type 17 example. Immunology. 2010; 129:147-53.
31. Crome SQ, Wang AY, Levings MK. Translational mini-review series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. Clin Exp Immunol. 2010; 159:109-19.
32. Sakaguchi S. Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005; 6:345-52.
33. Toldi G, Rigó J Jr, Stenczer B, Vásárhelyi B, Molvarec A. Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia. Am J Reprod Immunol. 2011; 66:223-9.
34. Toldi G, Saito S, Shima T, Halmos A, Veresh Z, Vásárhelyi B, et al. The frequency of peripheral blood CD4+ CD25high FoxP3+ and CD4+ CD25- FoxP3+ regulatory T cells in normal pregnancy and pre-eclampsia. Am J Reprod Immunol. 2012; 68:175-80.
35. NakashimaA, Ito M, Shima T, BacN D,Hidaka T, Saito S. Accumulationof IL-17-positive cells in decidua of inevitable abortion cases. Am J Reprod Immunol. 2010; 64:4-11.
36. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. J Reprod Immunol. 2012; 93:75-81.
37. Jasper MJ, Tremellen KP, Robertson SA. Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. Mol Hum Reprod. 2006; 12:301