



Original article

## The role of Th1 and Th2 cytokines among women with recurrent spontaneous miscarriage

M. Sharief<sup>a,\*</sup>, R. Mohammed<sup>b</sup>, W.S. Shani<sup>b</sup>

<sup>a</sup>Department of Gynecology & Obstetrics, College of Medicine.

<sup>b</sup>Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

\*Corresponding author; Department of Gynecology & Obstetrics, College of Medicine.

### ARTICLE INFO

#### Article history:

Received 01 July 2014

Accepted 06 July 2014

Available online 28 July 2014

#### Keywords:

Cytokines

Miscarriage

pregnancy

### ABSTRACT

The objective of this study was to compare the concentrations of Th1 (IFN- $\gamma$ , TNF- $\alpha$ ) and Th2 (IL-4, IL-10) cytokines in the serum of normal pregnant and recurrent spontaneous abortion (RSA) women in 1st trimester as well as in the normal fertile non pregnant women. In RSA women there was a significant increasing in the mean concentration of Th1 cytokines (TNF- $\alpha$  and IFN- $\gamma$ ) as well as in Th1/TH2 than normal pregnant and non pregnant women. In normal pregnant women there was significant increasing in the mean concentration of Th2 cytokines (IL-4 and IL-10). RSA was associated with increasing concentration of Th1 cytokines while successful pregnancy was associated with increase concentration of Th2 cytokines in the first trimester of pregnancy, indicating Th2 bias in normal pregnancy and a Th1 –bias in unexplained RSA.

© 2014 Sjournals. All rights reserved.

### 1. Introduction

The maternal immune system is in close contact with cells and tissue from the semi-allogenic fetus during pregnancy. Therefore, there must be specific mechanisms to moderate the maternal immune system so that the pregnant woman does not reject her own fetus. However, the pregnancy can be compromised by a number of

complications, such as threatened abortion, recurrent spontaneous miscarriage, preeclampsia, and preterm delivery (Raghupathy and Kalinka 2008)

Recurrent spontaneous miscarriage (RSA) can be defined as two or more pregnancy losses before 20 weeks of gestation. It has been claimed that RSA occurs in 1% of women who are trying to have children (<http://rba-online.com/recurrentloss.asp>). Identified causes of RSA include uterine malformation, anti-phospholipid antibodies, and cytogenetic anomalies, but these account for only about 20-50% of the causes of RSA. Immunological causes and consecutive immunological therapies are proposed if no other reasons for RSA are found (Stray-Pederson and Stray-Pederson, 1984).

Both normal pregnancy and pregnancy with complications have been viewed from the perspective of the Th1/Th2 paradigm. Th1 and Th2 cells are the major subsets of T-helper (Th) cells with different patterns of cytokine production and different roles in immune responses (Coffman RL and Romagnani S, 1999). Their productions are mutually antagonistic to each other; thus, an individual who produces a strong Th1 response usually tends to have a low Th2 response and vice versa (Raghupathy and Kalinka, 2008).

Several studies showed Th1-type (inflammatory) responses are weakened during pregnancy, while Th2 responses are augmented. Humoral immune responses are enhanced during normal pregnancy, while cell-mediated immune responses, such as delayed-type hypersensitivity, natural killer (NK) activity, responses to intracellular infections and the course of cell-mediated autoimmune disorders are down-regulated; suggesting Th-1 bias in pregnancy failure and Th-2 bias in successful pregnancy (Piccinni., 2005; Wegmann *et al.*, 1993 and Raghupathy, 1997).

Th-1 cells are involved in cell-mediated inflammation and produce pro-inflammatory cytokines mediate their effects via their characteristic cytokines tumour necrosis factor-beta (TNF- $\beta$ ), tumour necrosis factor- alpha (TNF- $\alpha$ ), interferon-gamma (IFN- $\gamma$ ) and Interleukin-2 (IL-2) (Piccinni., 2005). Hill *et al.*, had been shown in 1987 both of TNF- $\alpha$  and IFN- $\gamma$  inhibit embryonic and fetal development as well as inhibit proliferation of human trophoblast lines. In addition Yui *et al.*, 1994 found that both of them are cytotoxic to trophoblast cells and Knackstedt *et al.*, 2003 found that both of TNF- $\alpha$  and IFN- $\gamma$  cause inflammation and activate coagulation via up-regulating the novel prothrombinase-fgl2 and these are thought to be threaten in maintenance of pregnancy.

During normal pregnancy a shift from a Th1- to Th2 anti-inflammatory cytokines such as interleukin 4 (IL-4), IL-10, polarized immune response contributes to the survival of the fetus (Ramhor and Fainboim 2005).

IL-4 is the dominant factor for promoting growth and differentiation from the Th0 to the Th2 subtype, and directly inhibits the development of the Th1 cells (Skykes L. *et al.*, 2012)

IL-10 is called cytokine synthesis inhibitory factor because it inhibits cytokine synthesis by Th1 cell subset. It is produced by macrophage and dendritic cells and the cells that produced it are the major target cells for its inhibitory activities (Elgert 2009).

Despite showing a suppression of IFN- $\gamma$  and an increase in IL-10 during pregnancy compared to nonpregnant controls, Bates *et al.* 2002 showed no difference in IFN- $\gamma$ , IL-10, or IL-4 secretion in women who subsequently miscarried compared with those who went on to complete their pregnancy.

RSA is one of the most important complications of pregnancy in Iraq that increase remarkably during the last years. Most of the previous studies focusing on parasitic (Al-Hamdani and Mahdi 1997; Shani WS 2004) or viral (Al-Obaidi 2008; Sahdoon 2010) relation to RSA; little studies were done on immunological relation to RSA especially about the Th1/Th2 ratio either in the normal pregnant or RSA or fertile nonpregnant women. So this study tries to shot light about the associated of Th1, Th2 cytokines with normal pregnancy and RSA.

## 2. Materials and methods

### 2.1. Samples collection

Three ml of venous blood were collected by vein puncture in plain tube and serum isolated shortly thereafter from 80 women from January 2008 to March 2010.

39 of them were suffering from RSA in the first trimester (have 2 < consecutive spontaneous miscarriage) attending to (emergency unite of obstetrics and gynecology of Basrah maternity and children hospital) with vaginal bleeding for evacuation.

All the patients with RSA were subjected previously to the following tests TORCH {toxoplasmosis, rubella, cytomegalovirus (CMV) and herpes simplex virus (CMV)}, anti-nuclear, anti-phospholipids, anticardiolipin

antibodies, progesterone levels (>10 ng/mol), thyroid function (T3 between 0.9-2.5 Mlu/ml; T4 between 60-120 Mlu/ml) and glucose titration test.

A positive control group was 21 normal pregnant women, who had at least one or more child with no history of miscarriage, ectopic pregnancy, pre-term delivery or stillbirth. A negative control consist of 20 fertile non pregnant with normal menstrual cycle and were not affected by pre-existing clinical conditions such as diabetes, hypertension, or autoimmune diseases, none of them was under medication at the blood collection time. The age of RSA and normal women either pregnant or non pregnant ranged from (17-40) years. The blood samples were collected from these women as followed:-

- ❖ From aborted women: - The blood was taken at the time of miscarriage.
- ❖ Normal pregnant women: - The blood samples collected at the first trimesters.
- ❖ Fertile healthy non pregnant women: - The blood samples were taken at any time during their visiting.

## 2.2. Measurement of serum cytokines

Concentrations of the cytokines IL-4, IL-10, TNF- $\alpha$ , and IFN- $\gamma$  were measured in the serum of women enrolling in the study by using enzyme-linked immunosorbent assays (ELISA). All kits were provided from US Biological Company /USA. The manufacturers' protocols were followed for each kits and recombinant reference cytokine samples served as positive controls for calibration.

## 2.3. Statistical analysis

Statistical Package for Social Science (SPSS) was used to analyze the data. ANOVA and t-test were used to assess the significance of differences between groups.

## 3. Results

### 3.1. Characteristic of studied groups

The general characteristics of women considered in this study can be seen in table (1).The range of age was from (17-40) years, with the mean of age (1.3 $\pm$ 0.46) for RSA women, (1.2 $\pm$ 0.43) for pregnant women (positive control) and (1.4 $\pm$ 0.5) for fertile non pregnant women (negative control), there was no significant difference among the groups in relation to age.

The mean number of pregnancies for RSA (3.9 $\pm$ 1.3) was significantly higher (p<0.003) than those in the control groups +ve control (3  $\pm$  1.4) and -ve control ( 2. 85 $\pm$ 1.22).

On the other hand the mean number of alive babies was high in control groups +ve control (2.04  $\pm$  1.24) and -ve control (2.85 $\pm$ 1.22) rather than in the RSA women (0.41 $\pm$  0.67) with high significant difference (P<0.000) among the groups

**Table 1**

General characteristics of RSA, normal pregnant and nonpregnant women.

Characteristics	RSA	Normal pregnant (+ve control)	Non-pregnant (-ve control)	ANOVA
<b>No. of studied groups</b>	<b>39</b>	<b>21</b>	<b>20</b>	
Age				
<30	27	16	12	F=0.794
$\geq$ 30	12	5	8	P=0.456
Mean $\pm$ SD	1.3 $\pm$ 0.46	1.2 $\pm$ 0.43	1.4 $\pm$ 0.5	
Gravidity				
Range	3-7	2-6	1-6	F=6.32
Mean $\pm$ SD	3.9 $\pm$ 1.3	3 $\pm$ 1.4	2.85 $\pm$ 1.22	P=.003
Number of alive babies				
Range	0-2	2-6	1-5	F=44.47
Mean $\pm$ SD	0.41 $\pm$ 0.67	2.04 $\pm$ 1.24	2.85 $\pm$ 1.22	P=.000

### 3.2. Mean concentration of TNF-alpha in the serum of RSA, nonpregnant and normal pregnant women

The results associated with the mean concentration of TNF-alpha was summarized in table (2) in which there was a high significant differences in the mean concentration of TNF-alpha among three studied groups (p<0.000).

Highly significant ( $P < 0.000$ ) increasing in the mean concentration of TNF-alpha in RSA women were recorded ( $308.56 \pm 154.5$ ) in comparing with nonpregnant women ( $116.95 \pm 50.49$ ) and the normal pregnant women ( $90.19 \pm 49.9$ ).

In addition to that, the mean concentration of TNF-alpha in the normal pregnant women ( $90.19 \pm 49.9$ ) was decreased than the fertile nonpregnant women ( $116.95 \pm 50.49$ ) with no significant differences

**Table 2**

Mean concentration of TNF-alpha in the serum of RSA, nonpregnant and normal pregnant women.

Groups	No	Range	mean± SD	ANOVA
*RSA	39	84-630	308.56±154.5	df=2
**Non-pregnant	20	50-225	116.95±50.49	F=32.547
***Normal pregnant	21	29-200	90.19± 49.9	0.000
Total	125			

t-test \* $\chi^2$ \*\*\*0.000

\* $\chi^2$ \*\*\*0.000

\*\* $\chi^2$ \*\*\*0.802

### 3.3. Mean concentration of IFN-gamma in the serum RSA, nonpregnant and normal pregnant women

The mean concentration of IFN-gamma was increased in RSA women ( $239.38 \pm 71.48$ ) than both of normal fertile women either pregnant ( $135.05 \pm 35.56$ ) or nonpregnant ( $136.85 \pm 36.27$ ) with significant differences (0.008) for both.

As well as ,the mean concentration of IFN-gamma was decreased in the normal pregnant women ( $135.05 \pm 35.56$ ) than the fertile nonpregnant women ( $116.95 \pm 50.49$ ) with no significant differences

**Table 3**

Mean concentration of IFN-gamma in the serum of RSA, nonpregnant and normal pregnant women.

Groups	No	Range	mean± SD	ANOVA
*RSA	39	150-400	239.38±71.48	df=2
**Non-pregnant	20	75-212	136.85±36.27	F=33.69
***Normal pregnant	21	75-205	135.05± 35.56	0.000
Total	125			

t-test \* $\chi^2$ \*\*\*0.008

\* $\chi^2$ \*\*\*0.008

\*\* $\chi^2$ \*\*\*0.930

### 3.4. Mean concentration of IL-4 in the serum of RSA, nonpregnant and normal pregnant women

The results associated with the concentration of IL-4 were summarized in table (4) in which there was a high significant differences in the mean concentration of IL-4 among three studied groups ( $p < 0.000$ )

The mean concentration of IL-4 was increased in normal pregnant women ( $494.81 \pm 220.12$ ), which was significantly higher ( $P < 0.000$ ) than the mean number of RSA women ( $233.87 \pm 32.5$ ) and fertile nonpregnant women ( $213 \pm 39.03$ ). While there was no significant difference in the mean concentration of IL-4 between RSA and fertile nonpregnant women.

**Table 4**

Mean concentration of IL-4 in serum of RSA, nonpregnant and normal pregnant women.

Groups	No	Range	mean± SD	ANOVA
*RSA	39	165- 293	233.87±32.5	df=2
**Non-pregnant	20	142-279	213±39.03	F=20.705
***Normal pregnant	21	274- 975	494.81± 220.12	0.000
Total	125			

t-test \* $\chi^2$ \*\*\*0.000

\* $\chi^2$ \*\*\*0.485

\*\* $\chi^2$ \*\*\*0.000

**3.5. Mean concentration of IL-10 in the serum of RSA, nonpregnant and normal pregnant women**

In the case of the mean concentration of IL-10 , the mean concentration in the RSA (182.79±36.58) was lower than the normal pregnant women (334.24± 67.738) with significant difference (p<0.004) and fertile nonpregnant women (189.35± 48.65)with no significant difference.

Also there was no statistical difference in the mean concentration of IL-10 between fertile normal pregnant women and nonpregnant women.

**Table 5**

Mean concentration of IL-10 in the serum of RSA, nonpregnant and normal pregnant women.

Groups	No	Range	mean± SD	ANOVA
*RSA	39	119-251	182.79±36.58	df=2
**Non-pregnant	20	130-320	189.35± 48.65	F=70.88
***Normal pregnant	21	255- 472	334.24± 67.738	0.000
Total	125			

t-test \*X\*\*\*0.004  
 \*Y\*\*0.422  
 \*\*Y\*\*\*0.122

**3.6. Mean concentration of Th1/Th2 in the serum of RSA, nonpregnant and normal pregnant women**

The mean percentage of Th1/Th2 was increased in RSA women (1.33 ± 0.46) than both of normal fertile women either pregnant (0.34±. 0.21) or nonpregnant (0.64±. 0.19) with statistical differences (p<0.001).

The mean percentage of Th1/Th2 In fertile nonpregnant women was closely related to the mean value of normal pregnant women without statistical differences (p>0.05)

**Table 6**

Mean concentration of Th1/Th2 blood of RSA, nonpregnant and normal pregnant women.

Groups	No	Range	mean± SD	ANOVA
*RSA	39	0.8-2.63	1.33± 0.46	df=2
**Non-pregnant	20	0.34-1.23	0.64±. 0.19	F=58.904
***Normal pregnant	21	0.14-1	0.34±. 0.21	0.000
Total	125			

t-test \*X\*\*\*0.001  
 \*X\*\*0.001  
 \*\*X\*\*\*0.784

**4. Discussion**

Pregnancy requires physiologic adaptations in all maternal systems, including the immune system. This process is complex and includes modifications at different levels and compartments of the maternal immune system (Luppi, 2003).

Successful pregnancy is characterized by a shift toward Th2 type immune response and suppression of adaptive immune responses to ensure acceptance of the semi-allogenic fetal graft; While, susceptibility to recurrent miscarriage is probably mediated by Th1 type immune response with pronounced expression and secretion of pro-inflammatory cytokines like TNF-α and IFN-γ paralleled with decreased production of anti-inflammatory cytokines like IL-10 (Bermas and Hill 1997 and Karhukorpi 2005)

In this study, highly significant increased concentration of Th1cytokines (TNF- α and IFN- γ) as well as in Th1/Th2 were found at the first trimester of pregnancy in RSA women as compared with normal pregnant at the same stage and with the non pregnant women . While the concentration of anti-inflammatory (IL-4 and IL-10) cytokines in normal pregnant women were significant increase than RSA and nonpregnant women. These results were agreed with ( **Makhseed** et al., 2000;Rezaei and Dabbagh, 2002) who measured the concentration of the

cytokines in serum and with (Homes et al.,2003) in plasma. So the successful pregnancy is characterized by a shift toward Th2 type immune response and suppression of adaptive immune responses to ensure acceptance of the semi-allogenic fetal graft; While, susceptibility to recurrent miscarriage is probably mediated by Th1 type immune response with pronounced expression and secretion of pro-inflammatory cytokines like TNF- $\alpha$  and IFN- $\gamma$  paralleled with decreased production of anti-inflammatory cytokines like IL-10 (Bermas and Hill 1997).

The possible explanation for recurrent miscarriage is pro-inflammatory cytokines may convert NK cells into lymphokine-activated killer cells that have been shown to lyse trophoblast cells. Direct effects of type 1 cytokines may include the apoptosis of trophoblast cells by TNF- $\alpha$  and IFN- $\gamma$ , inhibition of secretion of the growth-stimulating cytokines from the uterine epithelium and cytokine induced activation of coagulation mechanisms, which may then lead to vasculitis affecting maternal blood supply to the implanted embryo (Raghupathy 2003).

In the experimental study done in mice by Chaouat et al. (1990), was found that injection of specific concentration of IL-2, TNF- $\alpha$  and IFN- $\gamma$  together would terminated normal pregnancy in these mice. As well as TNF- $\alpha$  is known to cause fetal expulsion due to uterine contraction or may even cause necrosis of implanted embryo (Raghupathy, 1997) and TNF- $\alpha$  is also reported to act along with the hormones and causes thromboses in the placenta during pregnancy resulting in miscarriage and its production is enhanced at the onset of labor and spontaneous abortion (Daher et al.,1999).

Th-2 to Th-1 shift in pregnancies may be due to one or more factors. The deficiency of some putative immunomodulatory molecules like PIBF, placental factors, IL-10 or TGF- $\beta$ 2 may be responsible. It is also likely that a balance between IL-12 (favouring Th-1 response) and IL-4 (favouring Th-2 response) determines the eventual outcome of the Th-1 – Th-2 dichotomy during an immune response (Walia et al., 2008).

## References

- Al-Hamdani, M.M., Mahdi, N.K., 1997. Toxoplasmosis among women with habitual abortion. *East. Med. Health. J.*, (2), 310-315.
- Bates, M.D., Quenby, S., Takakuwa, K., Johnson, P.M., Vince, G.S., 2002. Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss. *Human Reprod.*, 17 ( 9), 2439–2444.
- Bermas, B.L., Hill, J.A., 1997. Proliferative responses to recall antigens are associated with pregnancy outcome in women with history of recurrent spontaneous abortion. *clin. invest.*, 100(6), 1330-1334.
- Chaouat, G., Menu, E., Clark, D.A., Minkowsky, M., Dy, M., Wegmann, T.G., 1990. Control of fetal survival in CBA x DBA/2 mice by lymphokine therapy. *J. Reprod. Fertil.*, 89, 447.
- Cited by Pfeiffer, K.A., Fimmers, R., Engels, G., Van-der-Ven, H., 2001. The HLA-G is potentially associated with idiopathic recurrent spontaneous abortions. *Mol. Hum. Reprod.*, 7, 373–8.
- Coffman, R.L., Romagnani, S., 1999. (eds) *Redirection Th1 and Th2 subsets*. Springer-Verlag Berlin.
- Daher, S.; Fonseca, F.; Ribeiro, O.G.; Musatti, C.C. and Gerbase- DeLima, M. (1999). Tumor necrosis factor during pregnancy and the onset of labor and spontaneous abortion. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 83, 77-79.
- Definition of RSA (on line ) from (<http://rba-online.com/recurrentloss.asp>) last update October 27<sup>th</sup>, 2004. cited by Meka Aand Reddy B M (2006). Recurrent Spontaneous Abortions: An Overview of Genetic and Non-Genetic Backgrounds. *Int. J. Hum. Genet.*, 6(2), 109-117.
- Elgert, K.D., (ed) 2009. *Immunology : understanding the immune system* .2nd ed. John wiley and sons., 305-395.
- Hill, J.A., Haimovici, F., Anderson, D.J., 1987. Products of activated lymphocytes and macrophages inhibit mouse embryo development in vitro. *J. Immunol.*, 1, 139(7), 2250-2254.
- Homes, V.A., Wallace, J.M., Gilmore, W.S., Mcfaul, P., Alander, H.D., 2003. Plasma levels of the immunomodulatory cytokine interleukine -10 during normal human pregnancy :a longitudinal study. *Cytokine.*, 21(6), 265-269
- Karhukorpi, J., Laitinen, T., Tiilikainen, A.S., 1997. HLA-G polymorphism in Finnish couples with recurrent spontaneous miscarriage. *Br. J. Obstet. Gynaecol.*, 104,1212–1214.
- Knackstedt, M.K., Zenclussen, A.C., Hertwig, K., Hagen, E., Dudenhausen, J.W., Clark, D.A., Arck, P.C., 2003. Th1 cytokines and the prothrombinase fgl2 in stress-triggered and inflammatory abortion. *Am. J. Reprod. Immunol.*, 49(4), 210-20
- Luppi, P., 2003. How immune mechanisms are affected by pregnancy. *Vaccine.*, 28, 21(24),3352-7 .
- Makhseed, M., Raghupathy, R., Azizieh, F., Farhat, R., Hassan, N., Bandar, A., 2000. Circulating cytokines and CD30 in normal human pregnancy and recurrent spontaneous abortions. *Hum. Reprod.*, 15( 9), 2011-2017 .

- Piccinni, M.P., 2005. T Cells in Pregnancy. In Markert UR (ed): Immunology of Pregnancy. Chem Immunol . Basel. Karger., 89, 3-9 .
- Piccinni, M.P., 2005. T Cells in Pregnancy. In Markert UR (ed): Immunology of Pregnancy. Chem Immunol . Basel. Karger., 89, 3-9
- Raghupathy , R., 1997. Th1-type immunity is incompatible with successful pregnancy. Immunol. Today; 18:478-482
- Raghupathy, R., 1997. Th1-type immunity is incompatible with successful pregnancy. Immunol. Today., 18, 478-482.
- Raghupathy, R., 2003. The immunology of unexplained recurrent spontaneous abortion: cytokines as key mediators. Bulletin Kuwait inst. med. Specializat,, 2, 32-38
- Raghupathy, R., Kalinka, J., 2008. Cytokine imbalance in pregnancy complications and its modulation. Front. Biosc., 13, 985-994.
- Ramhor, R.E., Fainboim, L., 2005. New Actors for the Immunological Mechanisms Involved in the Materno-Fetal Tolerance. Current Women's Health Rev. 1, 15-20
- Rezaei, A., Dabbagh, A., 2002. T-helper (1) cytokines increase during early pregnancy in women with a history of recurrent spontaneous abortion. Med. Sci. Monit., 8(8),607-610
- Sahdoon, R.N., The association of acute human parvovirus B19 infection and spontaneous miscarriage in Basrah ,Iraq(2010).Msc thesis submitted to the college of medicine ,university of Basrah.
- Shani, W.S., 2004. Humoral and cellular immune response in women afflicted with toxoplasmosis. PhD thesis submitted to the college of Science. University of Basrah.
- Shimada, S., Iwabuchi, K., Kato, E.H., Morikawa, M., Sakuragi, N., Onoé, K., Minakami, H., Yamada, H., 2003. No difference in natural-killer-T cell population, but Th2/Tc2 predominance in peripheral blood of recurrent aborters. Amer. J. Reprod. Immunol., 50( 4), 334–339.
- Stray –Pederson, B., Stray- Pederson, S., 1984. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. Am. J. Obstet. Gynecol., 148, 140-146.
- Sykes, L., David, A., MacIntyre, X., Yap, J., Sathana, P., Tiong, G.T., Phillip R., Bennett., 2012. Changes in the Th1 : Th2 Cytokine Bias in Pregnancy and the Effects of the Anti-Inflammatory Cyclopentenone Prostaglandin 15-Deoxy- $\Delta$ 12,14-Prostaglandin J2. Mediators Inflamm., 416739.
- Walia, G.K., Mukhopadhyay, R., Saraswathy, K.N., Puri, M., Chahal , S.M.S., 2008. Immuno-Molecular Etiology of Recurrent Pregnancy Loss and the Anthropological Perspective. Int. J. Hum. Genet., 8 (1-2), 227-235.
- Wegmann ,T., Lin, H., Guilbert, L., Mosmann, T.R., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon. Immunol. Today., 14, 353–356 .
- Wegmann, T., Lin, H., Guilbert, L., Mosmann, T.R., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol. Today., 14, 353–356
- women with spontaneous abortion., 2003. J. Fac. Med. Baghdad., 50(4), 484-487.
- Yui , J., Garcia-Lloret, M., Wegmann, T.G., Guilbert, L., 1994. Cytotoxicity of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and gamma-interferon (IFN- $\gamma$ ) against primary human placental trophoblasts. Placenta., 15, 819–828