Italian Journal of
Gynæcology & Obstetrics
March 2017 - Vol. 29 - N. 1 - Quarterly - ISSN 2385 - 0868

Association of Maternal IL-17 and IL-23R Polymorphisms with Risk of Recurrent Spontaneous Abortion in Women in Saudi Arabia

Afrah Fahad Alkhuriji[1]

[1] Zoology Department, King Saud University, Saudi Arabia

ABSTRACT

Background: Interleukin 17 (IL-17) is a pro-inflammatory cytokine mainly secreted by activated TH-17 cells and involved in inflammatory immune responses. Interleukin 23 (IL-23) sustains the development of inflammation and is considered a check point.

Aim: the aim of the present study was to investigate the association between serum IL-17A, IL-17F and serum IL-23receptor variants in Saudi women with unexplained spontaneous recurrence of abortion (RSA).

Methods: a total of 200 women; 100 RSA-patients matched with 100 controls were enrolled sequentially. Genetic distributions of the two variants of IL-17A, IL-17F and IL-23R genes were detected by a real-time polymerase chain reaction using the Taqman probe method.

Results: results showed a significant difference of genetic distribution of IL-17F (7488 T/C) between RSA-patients and controls, with respect to homozygous TT (χ2=144, p=0.001), homozygous CC (χ2=113, p=0.001) and heterozygous TC (χ2=8.87, p=0.003) genotypes that were associated with a significant different frequency of the phenotypic allele T (χ2=65.35, p=0.001) and allele C (χ2=100.90, p=0.005), implying that IL-17F of RSA-group belongs to a different population and/or underwent a mutation in this group of Saudi Arabian women. The adjusted odd ratios were 0.01, 53.08 and 12.24 for TT, TC and CC genotypes, respectively. The odd ratios for the allele T and C were 0.05 and 35.53, respectively. Regarding IL-17A (G-197A) homozygous AA genotype was significantly more frequent in RSA-group (χ2=4.92, p=0.026). For IL-23 (1142 G/A), homozygous GG genes was significantly increased in RSA-patients (χ2=5.364.92, p=0.021).

Conclusions: based on these results it can be concluded that genetic variants of IL-17 and IL-23 are involved in the development of RSA in Saudi Arabian women.

Keywords: IL-17, IL-23, Genotyping, Genetic Variants, Recurrent of Spontaneous Abortion, Pregnancy Loss.

SOMMARIO

Introduzione: l'interleuchina 17 (IL-17) è una citochina pro-infiammatoria principalmente secreta dalle cellule Th-17 attivate e coinvolta nella risposta immunitaria infiammatoria. L'interleuchina 23 (IL-23) sostiene lo sviluppo dell'infiammazione ed è considerata un punto di controllo.

Obiettivo: l'obiettivo di questo studio è stato quello di indagare l'associazione tra IL-17A, IL-17F sieriche e le varianti sieriche del recettore dell'IL-23 nelle donne saudite con aborto spontaneo ricorrente sine causa (RSA).

Metodi: un totale di 200 donne; 100 pazienti con RSA e 100 controlli sono stati arruolati in modo sequenziale. Le distribuzioni genetiche delle due varianti dei geni di IL-17A, IL-17F e IL-23R sono state rilevate da una reazione a catena della polimerasi in tempo reale utilizzando la sonda Taqman.

Risultati: i risultati hanno dimostrato una differenza significativa della distribuzione genetica di IL-17F (7488 T/C) tra le pazienti con RSA e i controlli, con riferimento ai genotipi TT omozigoti (χ2=144, p=0,001), CC omozigoti (χ2=113, p=0,001) e TC eterozigoti (χ2=8.87, p= 0.003), che sono risultati significativamente associati con una frequenza diversa del fenotipo allele T (χ2 = 65.35, p=0,001) e allele C (χ2 = 100.90, p=0,005), il che implica che l'iL-17F del gruppo con RSA appartenga ad una popolazione diversa e/o ha subito nel tempo una mutazione in questo gruppo di donne saudite. L'odds ratio per i genotipi TT, TC e CC erano rispettivamente 0.01, 53.08 e 12.24. L'odds ratio per gli alleli T e C erano rispettivamente 0.05 e 35.53. Per quanto riguarda IL-17A (G-197A) il genotipo omozigote AA era significativamente il più frequente nel gruppo con RSA (χ2=4.92, p=0.026). Per IL-23 (1142 G/A), l'omozigosi GG era significativamente più alta nelle pazienti con RSA (χ2=5.364.92, p=0,021).

Conclusioni: sulla base di questi risultati si può concludere che le varianti genetiche di IL-17 e IL-23 sono coinvolte nello sviluppo di RSA nelle donne saudite.

Corresponding to Prof. Al-Said A. Haffor: saidhaffor@yahoo.com

DOI: 10.14660/2385-0868-57

INTRODUCTION

Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses, before the 20th week of gestation[1-2]. It was estimated by the World Health Organization that each year 42 million of miscarriages (22 million safely and 20 million unsafely) occur in the world population. Ministry of Health (MOH) in Saudi Arabia reported 274012 cases during the 2001-2009 period, that is considered very high.

Several factors have been implicated in the etiology of miscarriage, these include uterine abnormalities, infections, genetic factors, environmental factors, thrombophilia, endocrine, metabolic and immunologic maternal pathologies[3-5]. However, etiology remains unexplained in almost 50% of RSA cases.

The supposed mechanism of RSA related to uterine malformations is the poor blood supply and the sex hormone receptors deficiency of the septum, which causes abnormal uterine contraction with subsequent fetal wastage and abortion[6]. (Hussain, 2003). Sex steroid hormones, including estrogen and progesterone, have been considered as important substances to maintain pregnancy. Recently, some studies have clarified that the effect of these hormones is mediated by cytokines[7]. Gynecological infections, like bacterial vaginosis, and Chlamydia trachomatis are involved in RSA.[8] Also chromosomal abnormalities have been implicated in the etiology of RSA[9-10].

During pregnancy, the immunologic system plays a crucial role that ensures normal pregnancy development and prevent the development of complications. Pregnancy requires strict temporal regulation of maternal immune function to accommodate fetus growth. Early implantation is facilitated by immunologic processes to ensure adequate vascular remodeling and placental invasion and prevent fetal rejection[11-13]. Recently, immune pathways have been implicated in the pathophysiology of RSA. Immune tolerance of the fetal-placental unit and placental angiogenesis are mandatory for a successful pregnancy outcome[14-15].

In early pregnancies, women who miscarry have high plasma levels of pro-inflammatory cytokines, while levels of anti-inflammatory cytokines are lower compared to women who maintain their pregnancy[7,14,16-18]. Clearly maternal lymphocytes in the uterus are expected to be in a state of immunosuppression in order to accept embryos as semiallografts, hence, cytokines play an important role in this process. In human studies, dominant Th1 immune responses in peripheral blood lymphocytes have been documented, it reflects the systemic contribution of Th1 cytokines to RSA and in multiple implantation failures in IVF cycles[19-20]. Enhanced uterine expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interferon-gamma (IFN-γ), IL-1β, and IL-6 is associated with embryo loss[21-22]. Antiinflammatory cytokines such as IL-10 seem to protect against inflammation-induced miscarriage[23-24].

From implantation until the end of a term pregnancy, the interaction between pro- and anti-inflammatory cytokines (IL-10 seems to be the key-cytokine) is fundamental, to promote normal pregnancy outcomes. Recent findings in healthy pregnancies show a global reduction of proinflammatory cytokines, such as TNF-α, IL-1β, and IL- 6, and an increase of counterregulatory cytokines, such as IL-10.

In view of the studies summarized above, genetic variants of these cytokines are associated with an increased susceptibility to certain inflammatory and infectious diseases. Genetic polymorphisms are reported in various cytokines, including TNF-α, TNF-β, IFN- γ, IL-4, IL-10 and IL-6[3,22,25-26]. The hypotheses of genetic contribution to Th1 propensity has been raised, but there are limited evidences of the involvement of cytokines' genetic polymorphism in RSA.

It is often hard to understand the role of genetic contribution, especially in immune regulation during pregnancy, because multiple cytokines are involved in immunopathology and each cytokine may have multiple polymorphisms or variants[22].

Cytokines IL-17A and IL-17F are known as pro-inflammatory and IL-23 receptor (IL-23R) are known as a check point of inflammation, however, their genetic polymorphisms in the unexplained recurrent spontaneous abortion in Saudi Arabian women have not been explored yet.

The aim of the present study was to evaluate the association of IL-17 F, IL-17A and IL-23R polymorphisms in Saudi female patients with unexplained RSA, matched with a healthy control group.

MATERIALS AND METHODS:

Participants - Participants were 200 women aged 18-45 years, with unexplained RSA, consecutively referred to the abortion clinic of King Khaled University Hospital in Riyadh, Saudi Arabia. The protocol of the present study was

approved by the IRB at King Khalid University Hospital and by the Ethical Committee of King Saud University. An informed consent was obtained for each participant.

Study Design - A prospective case-control study was conducted using two female groups. Patients with unexplained recurrence of spontaneous abortion (RSA) were identified sequentially as case group, hence designated as RSA-group. A second group, served as control group. The inclusion and exclusion criteria for both groups were outlined as follows:

RSA- Group - hundred women age 18-45 years and BMI 25-30 with unexplained RSA attending the outpatient clinic for abortion, at the Department of Obstetrics and Gynecology at the King Khalid University Hospital of Riyadh. The inclusion criteria were: minimum of three miscarriages, negative anatomical, hormonal, infective (CMV, toxo, rubella, HBV, HCV, HIV), immunological and chromosomal tests for RSA. Women with RSA in whom the cause of miscarriages was known, were excluded from the study.

Control Group - The control group consisted of 100 healthy Saudi females, recruited from King Khalid University Hospital, with the same demographic characteristics (age 18-45 years and BMI 25-30), with no abortion in their anamnesis and at least two successful pregnancies.

DNA Quantification - DNA was extracted from whole periferal blood using "Puregene DNA purification kit". The concentration and purity of each DNA samples were determined using a spectrophotometer (NanoDrop 2000). From each sample 100 µl working DNA (50ng/ µl) will be labeled and stored at 4°C or – 20° C.

Genotyping Assays - The "TaqMan" assay was used in order to detect SNPs in DNA extracted from samples. Specific primers and probes for the TaqMan genotyping method were used (Applied Biosystems) for both SNPs and were used according to the manufacturer's instructions.

Statistical analysis - Descriptive measures and correlation matrix were generated to describe the bivariate linearity among variables of interests by Pearson Product moment correlation (r). CHI square test was carried out to determine the differences in frequency of the RSA and control group. The odd ratio was calculated to examine the association between genotype and the phenotype characteristics. P value of 0.05 was adopted as the level of significancy. All statistical analyses were conducted using SPSS program version 20.

RESULTS

Demographic and Clinical Characteristics of Patients - The results of independent t-Test showed that there were no significant differences ($p<0.05$) in mean age, height, weight and BMI among the two groups of patients (**Table 1**). However, the means of the number of pregnancies and the number of abortion were significantly ($p<0.05$) higher in the RSA-group, compared to the control group. On the contrary, the mean number of children was significantly ($p<0.05$) less in the RSA-group compared to the control group.

Table 1.
Results of Independent t-Test of Demographic and Clinical Features of RSA-group and Control Group.

Parameter	RSA-patients Mean±SEM	Control Mean±SEM	*P value*
Age (yrs)	33.20±0.629	33.10±0.733	0.085
Height (m)	156.92±0.814	158.48±0.546	0.970
Weight (kg)	72.90±1.821	70.50±1.517	0.642
BMI (kg/m²)	29.34±0.593	28.28±0.614	0.472
No. of pregnancies	6.40±0.325	3.60±0.171	0.0001*
No. of children	1.95±0.201	3.60±0.171	0.002*
No. of abortions	4.48±0.244	0	0

*p<0.05

Il-17A Gene - The prevalence of Il-17A gene (G-197A) polymorphism was determined for both groups. The frequency of allele A was 0.275 in RSA-patients and 0.18 in controls. The number of patients with G/G, G/A and A/A genotypes were 56 (56%), 33 (33%) and 11 (11%) in RSA-group, and 67 (67%), 30 (30%) and 3 (3%) in controls. Overall, the distribution of G/G homozygote and G/A heterozygote don't differ significantly among patients with unex-plained recurrent spontaneous abortions and healthy control subjects, (P= 0.109) and (p=0.647) respectively, but differs significantly regarding the distribution of A/A homozygote (P= 0.026). Results are summarized in **Table 2**.

Table 2.
IL-17A Genotype and Allele Frequencies in RSA-Group and Control group

IL-17A (-197) G/A						
Genotype	**Control No. (%)**	**RSA No. (%)**	**Control vs. Patients**			
			OR	**CI**	**χ^2**	***p-value***
GG	67 (67%)	56 (56%)	0.63	0.35 – 1.11	2.555	0.109
GA	30 (30%)	33 (33%)	1.15	0.63 – 2.09	0.209	0.647
AA	3 (3%)	11 (11%)	4.00	1.08 – 14.8	4.916	0.026
Total	100	100				
Allele	**Control (Freq)**	**RSA (Freq)**	**Control vs. Patients**			
			OR	**CI**	**χ^2**	***p-value***
G	0.82	0.725	0.58	0.31 – 1.09	2.899	0.088
A	0.18	0.275	1.45	0.84 – 2.48	1.803	0.179

Il-17F Gene - The prevalence of Il-17F gene (7488 T/C) polymorphism was determined for both groups. The frequencies of C alleles were 0.545 in RSA-patients and 0.03 in controls. The number of T/T, T/C and C/C genotypes were 5 (5%), 84 (84%) and 11 (11%) in RSA-patients, and 90 (90%), 9 (9%) and 1 (1%) in controls. Overall, the distribution of the various genotypes of Il-17 gene (+7488) T/C significantly differ among patients with RSA and healthy control subjects (P=0.001 and p=0.005 respectively).
Results are summarized in T**able 3**.

Table 3.
IL-17F Genotype and allele frequencies in RSA-patients compared to the control group.

IL-17F (7488 T/C)						
Genotype	**Control No. (%)**	**RSA No. (%)**	**Control vs. RSA**			
			OR	**CI**	**χ^2**	***p-value***
TT	90 (90%)	5 (5%)	0.01	0.01 – 0.03	144.8	**0.001**
TC	9 (9%)	84 (84%)	53.08	22.2 – 136.5	113.0	**0.001**
CC	1 (1%)	11 (11%)	12.24	1.55 – 96.68	8.865	**0.003**
Total	100	100				
Allele	**Control (Freq)**	**RSA (Freq)**	**Control vs. RSA**			
			OR	**CI**	**χ^2**	***p-value***
T	0.97	0.455	0.05	0.02 – 0.012	65.35	**0.001**
C	0.03	0.545	36.53	14.9 – 89.2	100.9	**0.005**

Il-23R Gene - The prevalence of Il-23R gene (1142 G/A) polymorphism was determined for both groups. The frequency of allele A was 0.535 in RSA-patients and 0.435in controls. The number of patients with G/G, G/A and A/A genotypes was 10 (10%), 73 (73%) and 17 (17%) in RSA-group, and 22 (22%), 69 (69%) and 9 (9%)in controls.
Overall, distribution of the various genotypes of Il-23 gene (+1142 G/A) differ significantly among RSA-patients and healthy control sub¬jects regarding G/G homozygosity (P= 0.02) and (p=0.058) for allele A distribution.
Results are summarized in **Table 4**.

Table 4.
IL-23R Genotype and Allele Frequencies in RSA-Patients and Control Groups

IL-23R (1142 G/A)						
Genotype	**Control No. (%)**	**RSA No. (%)**	**Control vs. RSA**			
			OR	**CI**	**χ^2**	***p-value***
GG	22 (22%)	10 (10%)	0.39	0.18 – 0.88	5.357	**0.021**
GA	69 (69%)	73 (73%)	1.21	0.66 – 2.24	0.389	0.533
AA	9 (9%)	17 (17%)	2.07	0.88 –4.90	2.829	0.092
Total	100	100				
Allele	**Control (Freq)**	**RSA (Freq)**	**Control vs. RSA**			
			OR	**CI**	**χ^2**	***p-value***
G	0.565	0.465	0.67	0.41 – 1.09	2.646	0.103
A	0.435	0.535	2.30	0.94 – 5.62	3.402	**0.058**

DISCUSSION AND CONCLUSION

Findings of the present study showed different genetic distribution of pro-inflammatory cytokines (IL-17A, IL-17F) and inflammatory mediator (IL-23R) in RSA-women in Saudi Arabia. These genetic variants might provide the early molecular causes of maternal immunological rejection of the fetus. It was reported that T helper 17 (Th17), T regulatory (Treg) cells and their cytokines are the main players of immunomodulation in peripheral blood lymphocytes during the early phase of pregnancy[27]. The percentage of TH17 cells correlates with IL-23, IL-6 and IL-17, so with their expression. IL-6 gene should be correlated with IL-17. It was shown that the levels of TH17 cells were relatively enriched in the decidua of RSA patients, suggesting that TH-17 cells could play a key role in pregnancy immunological rejection[28].

The cytokine IL-17 is secreted by TH17 cells and it's known to have a major role in angiogenesis and immune regulation.[29] IL-17 is localized in both cyto- and syncytio-trophoblasts[30] and its expression by CD4+ T-cells was found higher in RSA-patients compared with normal pregnancies[31-32] IL-23 is a pro-inflammatory cytokine that exerts an adverse effect on pregnancy[20].

Normally, during pregnancy, the normal dominant T-helper (TH)-1 that induces inflammatory immune response by TNF and interferon is switched to a TH2 that by interleukin IL-4 and IL-10 leads a particular immune response that permits induction of maternal immune tolerance to the allogenic fetus and assures a successful implantation[20,33].

Previous studies showed that the proportion of TH17 (CD4+IL-17A+) cells in peripheral blood and decidua were higher in RSA-patients than in normal pregnant women[34].

Regarding the context of pregnancy, Treg and TH-17 cells are two lymphocytes with opposing actions. In normal pregnancy, Treg cells prevent generation of an immune response against fetal tissues and a decrease in the number of Treg cells is associated with abortion[28,35-36]. In contrast, TH17 cells promote inflammation, increased levels of this lymphocyte – accompanied by a decrease in Treg cell levels – were reported to occur in association with unexplained RSA[37]. Determining regulatory mechanisms that suppress TH17 cells might lead to novel approaches in the prevention of RSA. IL-23 has been linked with TH17 cell response, mainly through its effects on the expansion and maintenance of these cells[38-39]. In addition,

IL-23 is also involved in the differentiation and maintenance of CD4+ TH17 and CD8+ TC17 cells[40-42]. IL-17 and IL-23 are inducers of inflammatory response mediated by TH17 cells and were found high in RSA-patients[18,28,35-37,43-46]. Considering that different genetic variants of the IL-23R have been repeatedly implicated in a number of other pathologies.
Some studies also indicated that the A allele frequency of R381Q was lower in ankylosing spondylitis (AS) patients than in healthy controls[42].

The main difference between the two groups in our study regard IL-17F, results showed a significant difference of genetic distribution (7488 T/C) between RSA-patients and controls, with respect to homozygous TT ($\chi 2$ = 144, p=0.001), homozygous CC ($\chi 2$ = 113, p=0.001) and heterozygous TC ($\chi 2$ = 8.87, p=0.003) genotypes. In association a significant different frequency of the phenotypic allele T ($\chi 2$ = 65.35, p=0.001) and allele C ($\chi 2$ = 100.90, p=0.005) were found. These results implying that IL-17F of RSA-group could belong to a different population and/or that underwent a mutation in this group of Saudi Arabian women.

Based on results of the present study, it can be concluded that the genetic variants of IL-17 and IL-23R could be involved in the development of unexplained RSA in Saudi Arabian women. Other studies and larger cohorts are necessary to confirm these results.

CONFLICT OF INTERESTS

The author has no conflict of interest.

ACKNOWLEDGEMENT

This research project was supported by a grant from the "Research Center of the Female scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

REFERENCES

1) Pandey M, Rani R, Agrawal S. **An update in recurrent spontaneous abortion**. Arch Gynecol Obstet 2005; 272(2):95-108.
2) Dehghani R, Klantar S, Seyed-Hasani, S. **Cytogenetic analysis in couples with recurrent spontaneous abortion**. Iran. J. Reprod Med 2005; 4:13–17.
3) Liu C, Wang J, Zhou S. **Association between-238 but not308 polymorphism of TNF and unexplained recurrent spontaneous abortion (URSA) in Chinese population**. Reprod. Biol. Endocrinol 2010; 8: 114–118.
4) Ciancimino L, Laganà AS, Chiofalo B, Granese R, Grasso R, Triolo O. **Would it be too late? A retrospective case-control analysis to evaluate maternal-fetal outcomes in advanced maternal age**. Arch Gynecol Obstet. 2014 Dec;290(6):1109-14.
5) Ciancimino L, Laganà AS, Imbesi G, Chiofalo B, Mancuso A, Triolo O. ***Evaluation of Maternal-Fetal Outcomes After Emergency Vaginal Cerclage Performed With Shirodkar-McDonald Combined Modified Technique***. J Clin Med Res. 2015 May;7(5):319-23.
6) Hussein S. **Habitual abortion: causes, diagnosis, and treatment**. Reviews in Gynaecological Practice 2003; 75–80
7) Saito S, Nakashima A, Shima T, Ito M. **Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy**. Am J Reprod Immunol. 2010 Jun;63(6):601-10.
8) Hillier S, Holmes K, **Bacterial vaginosis. In: K. Holmes, P. Sparling, P. Mardh: Sexually Transmitted Diseases, 3rd Edition**. New York: McGraw-Hill, 1999, 563-586.
9) Reindollar R. **Contemporary issues for spontaneous abortion**. Obstetrics and Gyncology. September 1, 2000; 27 (3): 541–554.
10) Lakovschek I, Strubd B and Ulm B. **Natural outcome of trisomy 13, trisomy 18, and triploidy after prenatal diagnosis**. American Journal of Medical Genetics Part A 2011; 155A(11):2626-33.
11) Wang J, Hao C, Yi L, Yin G, Bao S, Qiu L. **Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients**. J Reprod Immunol 2010; 84:164–170.
12) Wang W, Hao C, Qu Q, Wang X, Qiu L, Lin Q. **The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained early recurrent miscarriage**. Hum Reprod 2010b; 25:2591–2596.
13) Wang W, Hao C, Qu Q, Wang X, Qiu L, Lin Q. I**nterleukin-17 and leptin genes polymorphisms and their levels in relation to recurrent pregnancy loss in Egyptian females**. Hum Reprod. 2010; Oct;25(10):2591-6.
14) Piccinni M. **T-Cells in normal pregnancy and recurrent pregnancy loss**. Reprod. Biomed. Online 2006; 13:840–844.
15) Wu HX, Jin LP, Xu B, Liang SS, Li DJ. **Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17**. Cell Mol Immunol. 2014 May;11(3):253-62.

16) Lim K, Odukoya O, Ajjan R, Weetman A, Cooke I. **Profile of cytokine mRNA expression in peri-implantation human endometrium**. Mol Hum Reprod 1998; 4:77–81.
17) Raghupathy R: **The immunology of unexplained recurrent spontaneous abortion: Cytokines as key mediators**. Bull. Kuwait Inst Med Spec 2003; 2:32–38.
18) Lee S, Kim J, Hur S, Kim C, Na B, Lee M. **An imbalance in interleukin-17-producing T and Foxp3 (+) regulatory T cells in women with idiopathic recurrent pregnancy loss**. Hum Reprod. 2011; 26(11); 2964–2971.
19) Larid S, Tucherman, E, Cork B. **A review of immune cells and molecules in women with recurrent miscarriage**. Human Reprod. Update 2003 9; 163–174.
20) Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH, Lin QD. **The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained early recurrent miscarriage**. Hum Reprod. 2010 Oct;25(10):2591-6.
21) Liu C, Wang J, Zhou S, Wang B, Ma X. **Association between -238 but not -308 polymorphism of Tumor necrosis factor alpha (TNF-alpha)v and unexplained recurrent spontaneous abortion (URSA) in Chinese population**. Reprod Biol Endocrinol. 2010 Sep 28;8:114.
22) Bahadori M, Zarei S, Zarnani AH, Zarei O, Idali F, Hadavi R, Jeddi-Tehrani M. **IL-6, IL-10 and IL-17 gene polymorphisms in Iranian women with recurrent miscarriage**. Iran J Immunol 2014; 11(2):97–104.
23) Wang WJ, Hao CF, Yi-Lin, Yin GJ, Bao SH, Qiu LH, Lin QD. **Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients**. J Reprod Immunol. 2010 Mar;84(2):164-70
24) Hosseini S, Shokri F, Ansari Pour S, Jeddi-Tehrani M, Nikoo S, Yousefi M, Zarnani AH. **A shift in the balance of T17 and Treg cells in menstrual blood of women with unexplained recurrent spontaneous abortion**. J Reprod Immunol. 2016 Aug;116:13-22.
25) Roses RE, Xu S, Xu M, Koldovsky U, Koski G, Czerniecki BJ. **Differential production of IL-23 and IL-12 by myeloid-derived dendritic cells in response to TLR agonists**. J Immunol. 2008 Oct 1;181(7):5120-7.
26) Najafi S, Hadinedoushan H, Eslami G, Aflatoonian A. **Association of IL-17A and IL-17 F gene polymorphisms with recurrent pregnancy loss in Iranian women**. J Assist Reprod Genet. 2014 Nov;31(11):1491-6.
27) Williams Z. ***Inducing tolerance to pregnancy***. New England Journal of Medicine. 2012 Sep 20; 367: 1159–1161.
28) Lee SK, Kim JY, Lee M, Gilman-Sachs A, Kwak-Kim J. **Th17 and regulatory T cells in women with recurrent pregnancy loss**. Am J Reprod Immunol. 2012 Apr;67(4):311-8.
29) Mandal M1, Marzouk AC, Donnelly R, Ponzio NM. **Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells**. Am J Reprod Immunol. 2012 Apr;67(4):311-8.
30) Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, Tatiwat P, Kunngurn S, Searle RF. **Interleukin-17 expression in the human placenta**. Placenta. 2007 Jan;28(1):59-63.
31) Liu YS, Wu L, Tong XH, Wu LM, He GP, Zhou GX, Luo LH, Luan HB. **Study on the relationship between Th17 cells and unexplained recurrent spontaneous abortion**. Am J Reprod Immunol. 2011 May;65(5):503-11.
32) Ng SC, Gilman-Sachs A, Thaker P, Beaman KD, Beer AE, Kwak-Kim J. **Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy**. Am J Reprod Immunol. 2002 Aug;48(2):77-86.
33) Soares MJ. **The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface**. Reprod Biol Endocrinol. 2004 Jul 5;2:51.
34) Numasaki M1, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT. **Interleukin-17 promotes angiogenesis and tumor growth**. Am J Reprod Immunol. 2002 Aug;48(2):77-86
35) Saito S, Nakashima A, Ito M, Shima T. **Clinical implication of recent advances in our understanding of IL-17 and reproductive immunology**. Expert Rev Clin Immunol. 2011 Sep;7(5):649-57.
36) Kwak-Kim J, Yang KM, Gilman-Sachs A. **Recurrent pregnancy loss: a disease of inflammation and coagulation**. J Obstet Gynaecol Res. 2009 Aug;35(4):609-22.
37) antner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, Nanan R. **Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia**. J Immunol. 2009 Dec 1;183(11):7023-30.
38) Peng J, Yang XO, Chang SH, Yang J, Dong C. **IL-23 signaling enhances Th2 polarization and regulates allergic airway inflammation**. Cell Res. 2010 Jan; 20(1):62-71.
39) Sheibanie AF, Khayrullina T, Safadi FF, Ganea D. **Prostaglandin E2 exacerbates collagen-induced arthritis in mice through the inflammatory interleukin-23/ interleukin-17 Arthritis Rheum**. 2007 Aug; 56(8):2608-19.
40) Rahman P, Inman RD, Gladman DD, Reeve JP, Peddle L, Maksymowych WP. **Association of interleukin-23 receptor variants with ankylosing spondylitis**. Arthritis Rheum. 2008 Apr; 58(4):1020-5.
41) Sarin R1, Wu X, Abraham C. **Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses**. Proc Natl Acad Sci U S A. 2011 Jun 7;108(23):9560-5.
42) Duan Z, Pan F, Zeng Z, Zhang T, Wang S, Li G, Xu S, Xu J, Zhang L. **Interleukin-23 receptor genetic polymorphisms and ankylosing spondylitis susceptibility: a meta-analysis**. Rheumatol Int. 2012 May;32(5):1209-14.
43) Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y. **Increased expression of interleukin 17 in inflammatory bowel disease**. Gut. 2003;52:65–70.
44) Chen Z, Lin F, Gao Y, Li Z, Zhang J, Xing Y, Deng Z, Yao Z, Tsun A, Li B. **FOXP3 and RORγt: transcriptional regulation of Treg and Th17**. Int Immunopharmacol. 2011 May;11(5):536-42.
45) Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. **The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells**. Cell. 2006 Sep 22;126(6):1121-33.
46) Ouyang W, Kolls JK, Zheng Y. **The biological functions of T helper 17 cell effector cytokines in inflammation**. Immunity. 2008; 28:454–467.