CEOG Clinical and Experimental Obstetrics & Gynecology

Changes and clinical significance of peripheral blood helper T lymphocyte and natural killer (NK) cells in unexplained recurrent spontaneous abortion (URSA) patients after abortion and successful pregnancy

L.Y. Zhu[1], X. Chen[1], Z.Z. Xu[1], L. Xu[1], T. Mao[2], H. Zhang[1]

[1] Department of Obstetrics and Gynecology, the 2nd Affiliated Hospital of Soochow University, Suzhou
[2] Department of Clinical Laboratory, the 2nd Affiliated Hospital of Soochow University, Suzhou (China)

Summary

Objective: This study aims to investigate the number changes and the clinical significance of the peripheral blood T lymphocyte subsets and NK (natural killer) cells in unexplained recurrent spontaneous abortion (URSA) patients before and after abortion, as well as after successful pregnancy. *Materials and Methods:* Thirty-nine URSA patients (URSA-abortion group), among who 22 patients were followed up until the final successful parturition (URSA-pregnancy group), 31 normal-pregnancy (NP) cases and 25 normal non-pregnancy (NNP) control cases in which the peripheral blood T lymphocytes and subsets, B cells, and NK cells were assessed flow cytometry. *Results:* Compared with the URSA-pregnancy group and the NP group, the Th cells and NK cells of the URSA-abortion group increased ($p < 0.05$); compared with the NNP group, the total number of T cells decreased after the first, second, and third month of the URSA abortion ($p < 0.05$); Th cells decreased within one to six months of the URSA abortion ($p < 0.05$); proportion of NK cells was significantly higher in URSA patients ($p < 0.05$). *Conclusion:* The abnormal numbers of the peripheral blood T cell subsets and NK cells were related with the occurrence of URSA.

Key words: Recurrent spontaneous abortion; Pregnancy; Helper T lymphocyte; NK cells.

Introduction

Recurrent spontaneous abortion (RSA) refers to the loss of conception product or fetus twice or more than twice before the 28th week of gestation (body weight ≤ 1,000 g) [1], accounting for 1% to 5 % of the total pregnancy incidences. Clinical studies have found that the risk of spontaneous abortion of these patients on their subsequent pregnancy was up to 70% - 80% [2]. The repeated loss of fetus would bring great physical and psychological harm towards the pregnant women and families. Currently, there is lack of effective clinical control measures. The etiology is complex, which might be associated with fetal chromosomal abnormalities, maternal immune dysfunction, endocrine abnormalities, uterine anatomic abnormalities, infections, and environmental factors. After near 20 years of researches, it is gradually found that 80% of the unexplained recurrent spontaneous abortion (URSA) is related with the immune factors. Maternal-fetal interface is where the maternal tissues and the fetal components come into direct contact, the most important part of the immune response. Pregnancy is a homograft phenomenon. For the mother, embryo carries semi-allogeneic antigens, evading rejection by the maternal immune system during normal pregnancy. Several mechanisms contribute to immunoregulation of maternal-fetal interface. Abortion is a transplant rejection caused by the abnormal immune tolerance in maternal-fetal interface [3]. Currently, the known mechanism of the maternal-fetal interface immune tolerance is a complex regulatory system co-participated by the regulation of human leukocyte antigen and immune cells such as T lymphocytes, natural killer (NK) cells, macrophages, and dendritic cells [4]. T lymphocytes are the most important cell populations in the immune system, and divided into different subsets according to the different CD markers on their surface. The T lymphocyte subsets in normal body synergize with each other, maintaining the body's normal immune function. When an exception occurs in the numbers and functions of different lymphocyte subsets, the body might encounter immune disorders [5]. Vujaklija *et al.* found that the peripheral blood T cell subsets in URSA patients were abnormal [6]. NK cells are the large granular lymphocytes derived from the lymphoid hematopoietic stem cells in bone marrow, accounting for 10% -15% of the total number of human peripheral blood lymphocytes. NK cells could participate the non-specific immune re-

Revised manuscript accepted for publication October 14, 2013

Table 1. — *Comparison of general information among the three groups.*

Group	Cases	Age (years)	Pregnant time (n)	Max abortion times (n)	Pregnency duration (days)
URSA-abortion group	39	28.3 ± 3.22	2.8 ± 0.60	2.8 ± 0.60	65.6 ± 4.73
URSA-pregnancy group	22	27.4 ± 0.54	3.1 ± 0.40	2.1 ± 0.40	63.3 ± 4.98
NP group	31	26.6 ± 1.14	2.7 ± 0.46	0.8 ± 0.96	63.5 ± 5.12
NNP group	25	26.8 ± 2.34	2.6 ± 0.54	0.7 ± 0.34	-
P		0.340	0.094	0.004*	0.188

Note: * inter-group comparison, $p < 0.05$; - represented no data.

sponse, without the stimulation of antigen and the participation of antibodies, and could directly kill the virus-infected target cells and tumor cells. Peripheral blood NK cells could migrate into the uterine local immune microenvironment and differentiate into the decidual NK cells through certain hormones or chemokines, and thus leading to the occurrence of URSA [7]. Carlino *et al.* pointed out that there was a dynamic balance between the peripheral NK cells and the NK which migrated and differentiated into the decidua on the maternal-fetal interface, the abnormal content and function of NK cell were closely related with URSA [8].

In this study, the compared detection was performed among RSA patients, normal-pregnancy (NP) women and normal non-pregnancy (NNP) women, aiming to understand the differences of T cell subsets and NK cells, and long-term follow-up was also performed towards the RSA patients for the comparison of the similarities and differences before and after the abortion, or the pregnancy at the next pregnancy, in order to analyze the relationship of the changes with pregnancy, and to investigate the possible mechanism and the monitoring significance of the changes of T-cell subsets and NK cells in RSA pathogenesis, providing the clinical basis for the diagnosis and prevention of URSA.

Materials and Methods

General information

The conditions of URSA: 1) continuous spontaneous abortions twice or more than twice; 2) chromosome karyotype of the couple were normal; 3) the determination of reproductive hormone was normal, with no endocrine disease history; 4) without the infections of chlamydia trachomatis and mycoplasma urealytium; 5) without organic disease in genital tract; 6) autoimmune antibody was negative; 7) the examination of semen was normal [9].

Grouping

(1) URSA-abortion group: 39 URSA patients were included, who were diagnosed in this hospital in 2008-2011 and met the above criteria after a detailed history and system checks. The pregnancy was confirmed through B-ultrasonic gestational sac-probe, and the pregnancy period was 8~10 weeks. The B-dynamic monitoring showed that the embryo had no growth, no fetal heart or the fetal heart impulse disappeared. (2) URSA-pregnancy group: 22 patients were included, who were hospitalized from 2008 to 2011 and met the above conditions of URSA. The patients were diagnosed as early pregnancy, the B-ultrasound probe reached the gestational sac, and the B-dynamic monitoring exhibited the normal embryo growth and fetal heart. The patients were all successfully followed-up and gave birth. (3) NP group: 31 patients were collected at the same period who accepted the voluntary termination of pregnancy in the clinics of this hospital; the pregnancy time was 8-10 weeks. The patients had no history of spontaneous abortion and had a normal history of pregnancy and delivery, and had all confirmed existence of gestational sac embryo and fetal heart beating through B-ultrasound. (4) NNP group: 25 patients were collected at the same period, without a history of spontaneous abortion while with NP history, when the patients were diagnosed they were not pregnant. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the 2nd Affiliated Hospital of Soochow University. Written informed consent was obtained from all participants.

Specimen collection

URSA-abortion group: three ml peripheral blood was extracted before the uterus-cleaning, the 1st, 2nd, 3rd, 4th, 5th, and 6th month after the abortion; URSA-pregnancy group: three ml peripheral blood was extracted on the third month (eight to ten weeks) of pregnancy; NP group: three ml peripheral blood was extracted on the third month (eight to ten weeks) of pregnancy as the control group. The above blood samples were all detected by flow cytometry.

Detection indicators

T lymphocyte subsets: T cell ratio, helper T lymphocyte (Th) ratio, suppressor T lymphocyte (Ts) ratio, Th/Ts (CD4 + T/CD8 + T) ratio, B cells ratio, and NK cells ratio.

Detection method

After the conventional anticoagulation with heparin, three ml peripheral blood of each group underwent density centrifugation for the peripheral blood mononuclear cells with Ficoll-Hypaque method. The cell suspension was treated with CD3-PERCP/CD4-FITC/CD8-PE/ CD19-PERCP/CD56-PE/CD16-PE/CD3-FITC, then after the incubation and hyalinization, FC500 flow cytometer, with Cell Quest software, was used to sort and calculate the T cell subsets, including T cells ratio, Th cells ratio, Ts cells ratio, Th/Ts (CD4 + T/CD8 + T), B cells ratio of NK cells ratio.

Statistical analysis

SPSS17.0 software was used. The counting data were expressed as mean ± standard deviation, the comparisons of inter- and inner-group were performed with ANOVA, with $p < 0.05$ considered as statistically significant.

Results

General information

Except the maximum numbers of abortion, there were no significant differences among the other indicators of the four groups (Table 1).

Table 2. — *Comparison of peripheral blood immune cells in URSA-abortion group, URSA-pregnancy group and the NP group.*

Items	URSA-abortion group (n=39)	URSA-pregnancy group (n=22)	NP group (n=31)
T cells (%)	66.9 ± 6.96#	70.5 ± 5.67	70.4 ± 5.76
Th cells (%)	37.1 ± 6.87*#	35.0 ± 8.24	34.4 ± 5.30
Ts cells (%)	25.7 ± 6.44	25.7 ± 6.63	25.3 ± 5.26
Th /Ts	1.5 ± 0.63	1.7 ± 1.03	1.41 ± 0.36
B cells (%)	11.6 ± 4.23	10.9 ± 3.57	10.0 ± 5.64
NK cells (%)	15.2 ± 5.29* #	11.4 ± 4.73	11.9 ± 3.44

Note: *Compared with NP group, $p < 0.05$; #compared with URSA-pregnancy group, $p < 0.05$.

Table 3. — *Dynamic monitoring of various immune cells (n=39).*

Items	1st month after abortion	2nd month after abortion	3rd month after abortion	4th month after abortion	5th month after abortion	6th month after abortion	NNPgroup
T cells (%)	67.1±6.51*	69.1±5.95*	68.6±7.12*	70.6±4.10	70.3±6.15	70.7±6.42	72.8±6.17
Th cells (%)	36.0±7.64*	36.6±5.43*	35.0±6.83*	36.1±6.91*	36.0±7.34*	37.7±4.01*	41.2±8.53
Ts cells (%)	26.1±6.78	28.2±7.58	28.4±6.62	29.0±5.80	29.0±5.59	29.0±6.18	26.3±5.81
Th/Ts	1.51±0.69	1.40±0.46	1.31±0.43*	1.32±0.46*	1.43±0.80	1.33±0.36*	1.69±0.68
B cells (%)	11.3±3.86	11.5±4.58	10.6±3.74	11.2±3.73	12.4±2.43	11.7±3.31	11.7±2.45
NK cells (%)	13.9±4.09*	14.5±5.27*	14.4±5.87*	13.0±4.54	15.3±5.83*	14.2±6.15*	10.8±5.42

Note: *Compared with the NNP group, $p < 0.05$.

Comparison of immune cells of URSA-abortion group, URSA-pregnancy group and NP group

The comparison among the URSA-abortion group (before uterus-cleaning), the URSA-pregnancy group, and NP group revealed that, the ratios of Th cells and NK cells in the former significantly increased as 37.1 ± 6.87% and 15.2 ± 5.29%, with statistically significant difference ($p < 0.05$); compared with URSA-pregnancy group, the T cells of URSA-abortion group (before curettage) significantly decreased as 66.9 ± 6.96%, with statistically significant difference ($p < 0.05$, Table 2, Figure 1).

Dynamic monitoring of a variety of immune cells in URSA-abortion group (after uterus-cleaning)

Compared with the NNP group, the T cells ratios in URSA-aborting group, on the 1st, 2nd and 3rd month after abortion, significantly reduced, as 67.1 ± 6.51%, 69.1 ± 5.95%, and 68.6 ± 7.12%, respectively, with statistical significance ($p < 0.05$); the Th (CD4+ T cells) ratios of URSA-abortion group with 1~6 months after abortion significantly reduced, as 36.0 ± 7.64%, 36.6 ± 5.43%, 35.0 ± 6.83%, 36.1 ± 6.91%, 36.0 ± 7.34%, and 37.7 ± 4.01%, respectively, and the difference was statistically significant ($p < 0.05$); the Th/Ts (CD4+T/CD8+T cells) ratios on the 3rd, 4th, and 6th month after abortion decreased significantly, as 1.31 ± 0.43, 1.32 ± 0.46, and 1.33 ± 0.36, with statistically significant difference ($p < 0.05$); the NK cells ratios on the 1st and 2nd, 3rd, 5th, and 6th month after abortion significantly increased, as 13.9 ± 4.09%, 14.5 ± 5.27%, 14.4 ± 5.87%, 15.3 ± 5.83%, and 14.2±6.15%, respectively, with statistically significant difference ($p < 0.05$, Table 3, Figures 2, 3).

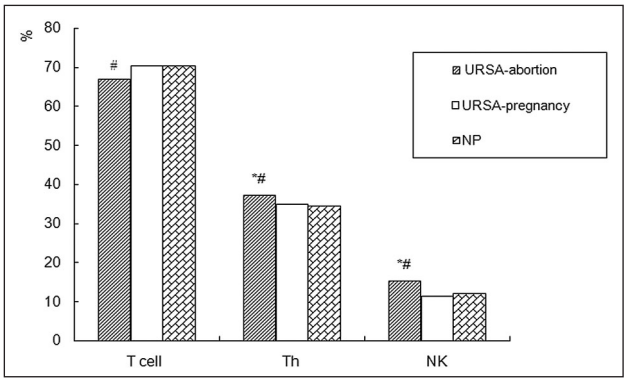

Figure 1. — Comparison of peripheral blood T lymphocytes, Th cells, and NK cells in URSA-abortion group, URSA-pregnancy group, and the NP group. *Compared with the NP group $p < 0.05$; # compared with the URSA-pregnancy group, $p < 0.05$.

Discussion

Currently, researches show that URSA is mainly related to immune factors. The immune tolerance on the maternal-fetal interface maintains the normal embryo not to suffer from maternal rejection; once this immune balance is broken, URSA would occur [10, 11].

CD4+T cells are auxiliary/inductive T lymphocytes (Th), which could mediating the cell immunity, and the increasing rate of Th could enhance the maternal immune function, and the immune rejection towards embryo would also increase, resulting in the pregnancy loss [12]. CD8+T cells belong to the killing/suppressive T cells (Ts), which could not only inhibit the B lymphocyte-mediated humoral immunity, but also in-

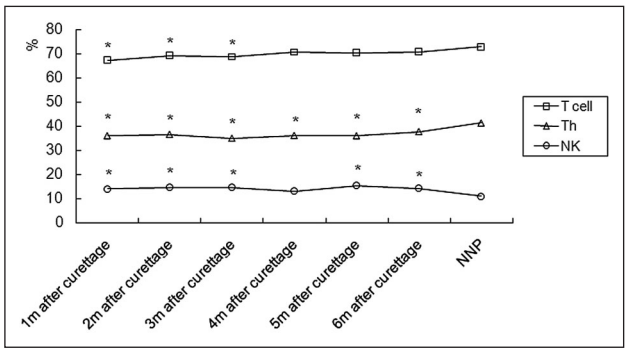

Figure 2. — Dynamic monitoring comparison of T lymphocytes, Th cells and NK cells in URSA patients 1~6 months after abortion. *Compared with the NNP group, $p < 0.05$.

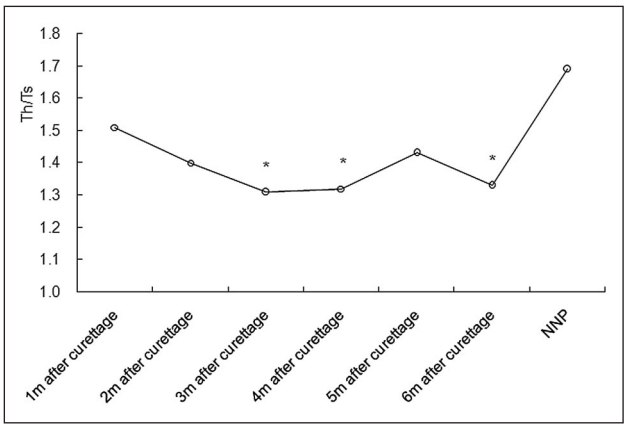

Figure 3. — Dynamic monitoring comparison of Th/Ts in URSA patients 1~6 months after abortion. *Compared with the NNP group, $p < 0.05$.

hibit CD4+T cell-mediated tardive hypersensitive reactions and proliferation, thus ensuring the embryonic semi-alloantigens would not be excluded. The above two factors would restrict each other mutually [13]. Under normal circumstances, the ratio of Th/Ts (CD4+T/CD8+T cells) would always maintain a dynamic equilibrium, no matter which one is too low or too high, the regulatory network would lose the balance, causing immune disorders. Warren *et al.* [14] found that Th/Ts <1. 0 or > 2.0 could be regarded as immune dysfunction, leading to the occurrence of URSA. The study revealed that compared with the URSA-pregnancy group and the NP group, the number of Th of the URSA-abortion group (before curettage) significantly increased, therefore it could be considered that the increasing of CD4+T cells might lead to the immune disorders, and thus participating in the occurrence of URSA. Carbone *et al.* [15] found that the CD4+T cells, T lymphocyte subsets in URSA patients, significantly elevated. Szpakowski *et al.* [16] also found that the ratio of CD4+T/CD8+T cells changed, the significant increasing of CD4+T cells would cause the pathological pregnancy. These studies supported the results of this study, showing that the occurrence of URSA was related with the CD4+T cells-mediated immune enhancement.

In this study, the dynamic monitoring found that Ts cells of URSA-pregnancy group did not change significantly after abortion, while after abortion, T lymphocytes within three months and CD4+T cells within six months would be significantly lower than those of the NNP group were, and showed a gradual trend to approach near the NNP levels, although the ratio of Th/Ts significantly reduced on the third, fourth, and sixth month after abortion, it was still in the normal range of immune regulation function (1.0-2.0), which might be because of the self-adjustment of body's immune system caused by the abnormally elevated T cells and Th cells after abortion. This abnormality would gradually return to normal levels with time. Therefore, clinical diagnosis could detect the peripheral immune cells, especially the results within three months of abortion, to consider whether the RSA was caused by immune factors.

In recent years, the impacts of NK cells have been increasingly emphasized on in URSA patients, especially that the decidual NK cells are considered to play an important role in the immune tolerance in the maternal-fetal interface. Different from the activation of T cells, during the antigen process proposed by T cell receptor-specific antigen presenting cells (APC), it is necessary to simultaneously recognize the MHC molecule, which would combine antigen and form the complex, to produce the T cell activation signal, while the decidual NK cells, different from the T cells, are non-MHC restricted, with the main roles of inhibiting the KIR to bind non-classical HLA-class antigens, inhibiting the cytotoxic activity of NK cells, secreting a variety of cytokines, and providing nutrition and protection towards the embryo [17]. Peripheral NK and decidual NK are in a state of dynamic equilibrium, but in the current study conditions, there is no safe and noninvasive method to detect the number of decidual NK cells in the pregnant status. Peripheral NK cells are relatively easy to sample, and could perform long-term tracking, which would facilitate the study. According to the surface markers on the peripheral NK cells' membrane, it could be divided into two subgroups, CD 56+CD 16+ (approximately 95%) and CD 56+CD 16- (about 5%). The former contains cytolytic granules, possessing the immune destruction and repulsion towards the embryo and the latter contains no cytolytic granules, and could produce a variety of cytokines, performing the immune protective and nutritional effects towards the embryo. Kim and Sachs [18] found that the peripheral blood NK cells in URSA patients and normal women did not change before and after pregnancy, and specific analysis towards the NK cell subsets revealed that peripheral blood CD56+CD16+ NK in URSA significantly increased [19]. In this study, compared with URSA-pregnancy group and the NP group, the NK cells of URSA-abortion group (before uterus-cleaning) was up to 15.26 ± 5.29%,

with significant difference, which was presumably considered as the performance of the maternal enhanced killing effects towards the embryo. Through dynamic monitoring, it was found that, within six months after abortion, NK cells significantly increased than the NNP group, and exhibited an upward trend, after considering that it would a longer period for NK cells to return to normal levels than Th cells after abortion. This study showed that the peripheral blood NK cells of the NP group and the URSA-pregnancy group were less than 12%, while > 12% in URSA-abortion group before abortion and six months after abortion. Beer *et al.* [20] and Karami *et al.* [21] found that the patients whose NK cells increased < 12%, usually could maintain the pregnancy to term, Paparistidis *et al.* [22] also found that the increasing contents of the peripheral NK cells in RSA patients five days after pregnancy termination could indicate that the last abortion was likely to be an immune abortion, and the patients with peripheral blood NK cells >12% would have nearly 90% probability of the occurrence of immune abortion, Yoo *et al.* [23] found that high concentrations of NK cells indicated that the subsequent pregnancy might still occur spontaneous abortion. It was considered that the detection of the peripheral blood NK cells level changes could diagnose whether the last abortion was immunity abortion or not, and could forecast the outcome of next RSA pregnancy.

In addition, in the present study, patients in the URSA-pregnancy group had a successful pregnancy and gave birth; compared with NP group, it was found that there was no significant difference in the immune cells, indicating that when the peripheral blood Th cells and NK cells of URSA patients returned to the normal levels, it might be highly likely to get pregnancy and give birth. Therefore, the clinical treatment should focus on how to reduce the peripheral blood Th cell and NK cell levels of URSA patients in order to achieve the therapeutic effect of URSA.

References

[1] Lathi R.B., Gray Hazard F.K., Heerema-McKenney A., Taylor J., Chueh J.T.: "First trimester miscarriage evaluation". *Semin. Reprod. Med*., 2011, *29*, 463.

[2] Shina A., Carp H.J.: "Recurrent pregnancy loss - beyond evidence based medicine". *Gynecol. Endocrinol*., 2012, *28*, 991.

[3] Krzymowski T., Stefańczyk-Krzymowska S.: "Advances in understanding the physiological mechanism of maternal immune tolerance to the embryo". *Reprod. Biol*., 2012, *12*, 265.

[4] Apps R., Sharkey A., Gardner L., Male V., Kennedy P., Masters L. *et al*.: "Ex vivo functional responses to HLA-G differ between blood and decidual NK cells". *Mol. Hum. Reprod*., 2011, *17*, 577.

[5] Prado-Drayer A., Teppa J., Sánchez P., Camejo M.I.: "Immunophenotype of peripheral T lymphocytes, NK cells and expression of CD69 activation marker in patients with recurrent spontaneous abortions, during the mid-luteal phase". *Am. J. Reprod. Immunol*., 2008, *60*, 66.

[6] Vujaklija D.V., Jericevic B.M., Laskarin G., Dominovic M., Tijanic T., Medancic S.S.: "Immunoregulation by Cytolytic Pathways, Mucins and Progesterone at the Maternal-Fetal Interface". *Adv. Neu. Bio*., 2011, *2*, 31.

[7] Kalkunte S., Chichester C.O., Gotsch F., Sentman C.L., Romero R., Sharma S.: "Evolution of non-cytotoxic uterine natural killer cells". *Am. J. Reprod. Immunol*., 2008, *59*, 425.

[8] Carlino C., Stabile H., Morrone S., Bulla R., Soriani A., Agostinis C. *et al*.: "Recruitment of circulating NK cells through decidual tissues a possible mechanism controlling NK cell accumulation in the uterus during early pregnancy". *Blood*, 2008, *111*, 3108.

[9] Dokouhaki P., Moghaddam R., Rezvany M., Ghassemi J., Novin M.G., Zarnani A. *et al*.: "Repertoire and clonality of T-cell receptor beta variable genes expressed in endometrium and blood T cells of patients with recurrent spontaneous abortion". *Am. J. Reprod. Immunol*., 2008, *60*, 160.

[10] Friebe A., Arck P.: "Causes for spontaneous abortion: what the bugs 'gut' to do with it"? *Int. J. Biochem. Cell. Biol*., 2008, *40*, 2348.

[11] Practice Committee of the American Society for Reproductive Medicine.: "Evaluation and treatment of recurrent pregnancy loss: a committee opinion". *Fertil. Steril*., 2012, *98*, 1103.

[12] Sasaki Y., Sakai M., Miyazaki S., Higuma S., Shiozaki A., Saito S.: "Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases". *Mol. Hum. Reprod*., 2004, *10*, 347.

[13] Norton M.T., Fortner K.A., Oppenheimer K.H., Bonney E.A.: "Evidence that CD8 T-cell homeostasis and function remain intact during murine pregnancy". *Immunology*, 2010, *131*, 426.

[14] Warren J.E., Silver R.M.: "Genetics of pregnancy loss". *Clin. Obstet. Gynecol*., 2008, *51*, 84.

[15] Carbone J., Gallego A., Lanio N., Navarro J., Orera M., Aguaron A. *et al*.: "Quantitative abnormalities of peripheral blood distinct T, B, and natural killer cell subsets and clinical findings in obstetric antiphospholipid syndrome". *J. Rheumatol.*, 2009, *36*, 1217.

[16] Szpakowski A., Malinowski A., Cieślak J., Nowak M., Wilczyński J.R., Banasik M. *et al*.: "Influence of paternal lymphocyte immunization on the selected subpopulations of peripheral blood lymphocytes in women with recurrent spontaneous abortions of unknown etiology". *Ginekol. Pol*., 2003, *74*, 288.

[17] Morandi F., Ferretti E., Castriconi R., Dondero A., Petretto A., Bottino C. *et al*.: "Soluble HLA-G dampens CD94/NKG2A expression and function and differentially modulates chemotaxis and cytokine and chemokine secretion in CD56bright and CD56dim NK cells". *Blood*, 2011, *118*, 5840.

[18] Kim K.J., Sachs G.A.: "Clinical implication of natural killer cells and reproduction". *Am. J. Reprod. Immunol*., 2008, *59*, 388.

[19] Emmer P.M., Nelen W.L., Steegers E.A., Hendriks J.C., Veerhoek M., Joosten I.: "Peripheral natural killer cytotoxicity and CD56posCD16pos cells increase during early pregnancy in women with a history of recurrent spontaneous abortion". *Hum. Reprod*., 2000, *15*, 1163.

[20] Beer A.E., Kwak J.Y., Ruiz J.E.: "Immunophenotypic profiles of peripheral blood lymphocytes in women with recurrent pregnancy losses and in infertile women with multiple failed in vitro fertilization cycles". *Am. J. Reprod. Immunol*., 1996, *35*, 376.

[21] Karami N., Boroujerdnia M.G., Nikbakht R., Khodadadi A.: "Enhancement of peripheral blood CD56(dim) cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure". *J. Reprod. Immunol*., 2012, *95*, 87.

[22] Paparistidis N., Papadopoulou C., Chioti A., Papaioannou D., Tsekoura C., Keramitsoglou T. *et al*.: "How valuable is measurement of peripheral blood natural killer cells at the time of abortion"? *Am. J. Reprod. Immunol*., 2008, *59*, 306.

[23] Yoo J.H., Kwak-Kim J., Han A.R., Ahn H., Cha S.H., Koong M.K. *et al*.: "Peripheral blood NK cell cytotoxicities are negatively correlated with CD8(+) T cells in fertile women but not in women with a history of recurrent pregnancy loss". *Am. J. Reprod. Immunol*., 2012, *68*, 38.

Address reprint requests to:
H. ZHANG, M.D.
Department of Obstetrics and Gynecology,
The 2nd Affiliated Hospital of Soochow University,
No 1055 San Xiang Road,
Suzhou 215004 (China)
e-mail: hongzhangdoc@126.com