Journal of Animal and Veterinary Advances 9 (16): 2194-2199, 2010
ISSN: 1680-5593

The Effects of IFN-γ on Expression of IL-18 in Uterus and Ovaries and IL-18 Levels in Peripheral Blood of Abortion Rats

[1,2]Si Li-Fang, [1]Chen Shu-Lin, [2]Cheng Xiang-Chao, [1]Qing Su-Zhu, [3]Si Xian-Li,
[1]Zhang Xiao-Ying, [1]Wang Jin-Yi, [1]Xu Yong-Ping and [1]Fan Guang-Li
[1]College of Veterinary Medicine, Northwest A and F University, Yangling, Shaanxi 712100, China
[2]College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan 471002, China
[3]Luoyang, Henan 46 School, Luoyang, Henan 471002, China

Abstract: To explore the immunological regulatory mechanisms of IFN-γ on IL-18 and its effect on pregnancy, the dynamic expression of mRNA and cellular localization of protein for IL-18 in the early pregnant rats after the injection of different doses of IFN-γ were observed by RT-PCR and immunohistochemistry. ELISA assay was also applied to investigate the effect of exogenous IFN-γ on the level of IL-18 in the peripheral blood. The results indicated that IL-18 mRNA expression level was the highest in uterus and ovaries of normal pregnancy group and the lowest in abortion model group; compared with the abortion model group, the IL-18 mRNA expression level was up-regulated both in 2.4 and 2.8 IU IFN-γ dose group. Howere, no significant difference can be seen between 2 IU IFN-γ dose group and the normal pregnancy group ($p>0.05$). Furthermore, IL-18 protein was strongly expressed in the normal pregnant group and 2IU IFN-γ group. ELISA results showed that the level of IL-18 in peripheral blood of abortion model was the lowest and it was extremely significantly different from normal pregnancy group and 2 IU IFN-γ group ($p<0.01$).

Key words: IL-18, IFN-γ, uterus, ovary, abortion, rat

INTRODUCTION

As a newly discovered cytokine, interleukin (IL)-18 plays an important role in anti-infection and allergic disease regulation (Boraschi and Dinarello, 2006; Tucci *et al*., 2008; Guan *et al*., 2009). Besides, it has some important effects on reproduction and immunoregulation as well (Ledee-Bataille *et al*., 2004; Gao and Hong, 2008). It was reported that pregnancy was a special Th2 phenomenon and the cytokines will be changed from cytokines Th1-Th2 in the peripheral blood cycle. This Th2 phenomenon came to the peak during the medium-term of pregnancy. As a kind of cytokines participated in many body immunological response, IL-18 play several important role in the process of pregnancy (a kind of semi-allograft transplant process). For example, IL-18 may regulate the activation of uterine NK cells and regulate Th1 and Th2 microenvironment-type immune response dependended cytokines (Guilbert, 1996). Sakai found out that the level of IL-18/ IL-12, both of which are secreted by monocytes in peripheral blood was higher in normal pregnant women than that of non-pregnant women and the enhancement of the Th2 advantage could be induced by the increased secretion of IL-18 or decreased secretion of IL-12 and it had better effects on maintaining pregnancy. Ledee-Bataille *et al*., 2004 found that the embryo implantation could be forecasted by the existence of IL-18 in the uterine. Therefore, there was a close relationship between IL-18 and pregnancy. The data demonstrated that immunoregulations depended on IFN-γ *in vivo* (Xie *et al*., 2005; Leng *et al*., 2008). IFN-γ, a Th1 type cytokine is critical in pregnancy. The prior studies had shown that super-physiological dose of IFN-γ had an anti-fertility effect (Cao *et al*., 1999). Meanwhile, it has been reported that the injection of a low dose of IFN-γ to pregnant rats would not raise the abortion rate but would enhance the ability of anti-toxoplasma infection (Jin *et al*., 2005).

The influence of the IFN-γ injection on immunoregulation and pregnancy has not been reported yet. In this study, PT-PCR, immunohistochemistry and ELISA technology were used to investigate the expression of IL-18 in uterus, ovaries and peripheral blood after the injection of exogenous IFN-γ to rats during later period of embryo implantation. In hope of knowing about the reason and pathogenesis of abortion from the immunological point of view, the data presented in this study provided new ideas for abortion and infertility treatment and presented a basis for the effects of cytokine network in pregnancy.

Corresponding Author: Chen Shu-Lin, College of Veterinary Medicine, Northwest A and F University, Yangling, Shaanxi 712100, China

MATERIALS AND METHODS

Experimental animals: Healthy female SD rats were purchased from the experimental animal center in the Fourth Military Medical University locating in Xi'an, Shaanxi Province, PR. China. SD rats weighted 240-250 g were used in this experiment. After a period of 1 week feeding, the rats were mated based on the estrus confirmation by vaginal smears. The day on which a positive vaginal smear was found it was considered as D1 of gestation and from that day on pregnant rats were housed individually.

Sample collection: The pregnant rats were divided into 6 groups (10 rats per group): normal pregnant group (pregnant on the 9th day), normal non-pregnant group (estrus female only), abortion model group (mifepristone 4 mg kg^{-1} day) at the 7th, 8th day), abortion model+exogenous injection of IFN-γ 2 IU group, 2.4 IU group and 2.8 IU group. For the abortion model+exogenous of IFN-γ 2 IU group, vaginal wall intramuscular injection of IFN-γ2, 2.4 and 2.8 IU g^{-1}, mifepristone gavage in the 7 and 8th day, the rats were sacrificed on D9. Drug treatment and doses referred to (Liu *et al*., 2002a, b).

The ovary and uterus of each group was obtained after cesarean section, the mesenteric tissues were taken away and washed 3 times with sterile PBS and frozen at -80°C. Rats used for the immunohistochemistry of Streptavidin-Peroxidase (SP) were anesthesiaed by 100 mL L^{-1} chloral hydrate then their chests were opened and the blood was cleaned by saline via the aorta at 37°C. After perfusion with 40 mL L^{-1} paraformaldehyde phosphate buffer solution (pH 7.4) at 4°C, the ovary and uterus were fixed for 48 h in the same fixative. Then the tissues were dehydrated in gradient ethanol, cleared by xylene and embedded by paraffin. Three sets of the serial 5 μm thickness paraffin sections were obtained for IL-18 immunohistochemical staining, staining with Hematoxylin and Eosin for detecting the position of positive cells and negative control group, respectively. ELISA detection: blood obtained from the rats heart on D9 was kept at 37°C for 1 h, then overnight at 4°C. Serum was separated and kept in different EP tubes at -20°C.

Total RNA extraction and RT-PCR: The total RNA of uterus and ovaries collected from each test group was extracted by TRIzol-Phenol-Chloroform method and dissolved in RNase-free water. The samples obtained were quantified by absorbance at 260 nm. Amplification of IL-18 and β-actin genes were performed by RT-PCR using an Access RT-PCR System Kit (Promega, USA). Amplification of specific fragments and anti-transcriptions were carried out in the same tube. The following primers were used: the forward primer of IL-18: 5'-AGGTTATCATAAGGCTCGT-3', the reverse primer of IL-18: 5'-GGCTGTGACCCTATCTGTG-3'. The amplication was 357 bp. The primers for β-actin were the forward primer: 5'-GCACCACACTTTCTACAAT-3', the reverse primer: 5'-CTGGGTCATCTTT TCACG-3', the length of the PCR fragment was 107 bp. The cycling protocol of the RT-PCR for IL-18 was 94°C for 5 min, 94°C for 45 sec (35 cycles), 54°C annealing for 45 sec, 72°C for 45 sec with a final extension at 72°C for 10 min. The protocol for the amplification of β-actin was the same as IL-18. In order to confirm the specificity of amplification, three kinds of control were used in each group (namely, nuclease-free water without reverse transcriptase, PCR without reverse transcription) while other conditions remained. The PCR products were detected by electrophoresis in 2.0% agarose gel. The A value of the electrophoretogram was statistically analyzed by Quantity One software (Bio-Rad Inc) after proofreading to that of β-actin. The data from each group was described by Mean±Standard Deviation, and performed by the Normal test before statistical analysis. T test were used to compare the differences among these data, $p<0.05$ and $p<0.01$ indicate significant difference. The analysis above was completed by statistical software SPSS 11.5.

Procedures of the immunohistochemistry and the results of observation: The procedures of immunohistochemistry SP method was performed by the immunohistochemical kit according to the manufacturer's protocol. Analysis of the results: 10 different magnitude microscopic vision (×400) from the same position of the different ovaries and uteruses were choose and then analyzed by Jiangsu Jetta high-resolution image analysis system, the mean of the staining degree of the positive product for each vision (which was denoted by the average optical density) and the positive area were calculated (Hu and Gui, 2004). The mean level of the relative expression (μ^2) were calculated via the following formula: μ^2 = Multiples of light microscope x Mean positive intensity x area of the positive 260000) among which 260000 was pixel.

Detection of levels of IL-18 level in serum by ELISA method: The ELISA experiments were performed following the instructions of ELISA kit and the OD values were measured by automatic microplate reader at 450 nm. Standard curve was drawn using the density of standard products and OD values as abscissa and vertical axis, respectively. Then the density of the sample can be found through the OD value in the standard curve.

RESULTS

The effects of IFN-γ on IL-18 mRNA in ovary and uterus of pregnant rats: Expression of IL-18 mRNA could be detected in the ovary of rats in each experimental group.

Table 1: The expression of IL-18 mRNA in the ovary and uterus of rats from different experimental groups (Mean±SD)

Parts	Normal pregnacy group	Non-pregnancy group	Abortion group	2 IU g^{-1} bodyweight	2.4 IU g^{-1} bodyweight	2.8 IU g^{-1} body weight
Ovary	2.41±0.024	1.75±0.006*	0.54±0.001**	2.39±0.009	1.79±0.003*	0.87±0.014**
Uterus	2.13±0.026	1.42±0.003**	0.43±0.002**	2.09±0.018	0.94±0.008**	0.75±0.007**

*Means significantly difference compared with normal pregnant group ($p<0.05$); **Means extremely significantly difference compared with normal pregnant group ($p<0.01$)

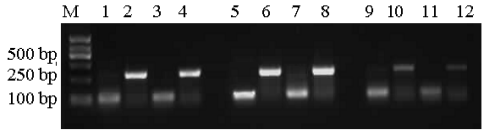

Fig. 1: The mRNA expression of β-actin and IL-18 in the ovary and uterus of rats from different experimental groups. Lane M :2 kb DNA ladder marker Lane 1: PCR amplification product of β-actin in ovary in 2.4 IU group; Lane 2: PCR amplification product of IL-18 mRNA in ovary in 2.4 IU group; Lane 3: PCR amplification product of β-actin in uterus in 2.4 IU group; Lane 4: PCR amplification product of IL-18 mRNA in uterus in 2.4 IU group; Lane 5: PCR amplification product of β-actin in ovary in non-pregnancy group; Lane 6: PCR amplification product of IL-18 mRNA in ovary in non-pregnancy group; Lane 7: PCR amplification product of β-actin in uterus in non-pregnancy group; Lane 8: PCR amplification product of IL-18 mRNA in uterus in non-pregnancy group; Lane 9: PCR amplification product of β-actin in ovary in abortion group; Lane 10: PCR amplification product of IL-18 mRNA in ovary in abortion group; Lane 11: PCR amplification product of β-actin in uterus in abortion group; Lane 12: PCR amplification product of IL-18 mRNA in uterus in abortion group

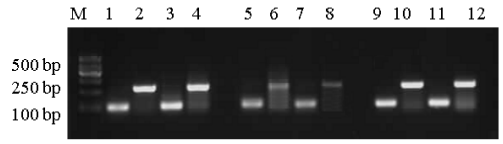

Fig. 2: The mRNA expression of β-actin and IL-18 in the ovary and uterus of rats from different experimental groups. Lane M : 2 kb DNA ladder marker Lane 1: PCR amplification product of β-actin in ovary in 2 IU group; Lane 2: PCR amplification product of IL-18 mRNA in ovary in 2 IU group; Lane 3: PCR amplification production of β-actin in uterus in 2 IU group; Lane 4: PCR amplification product of IL-18 mRNA in uterus in 2 IU group; Lane 5: PCR amplification product of β-actin in ovary in 2.8 IU group; Lane 6: PCR amplification production of IL-18 mRNA in ovary in 2.8 IU group; Lane 7: PCR amplification product of β-actin in uterus in 2.8 IU group; Lane 8: PCR amplification product of IL-18 mRNA in uterus in 2.8 IU group; Lane 9: PCR amplification product of β-actin in ovary in normal pregnant group; Lane 10: PCR amplification product of IL-18 mRNA in ovary in normal pregnant group; Lane 11: PCR amplification product of β-actin in uterus in normal pregnant group; Lane 12: PCR amplification product of IL-18 mRNA in uterus in normal pregnant group

However, the expression state was different. IL-18 mRNA expression in the ovary of normal pregnant group was the highest and was the lowest in abortion group. The expression of IL-18 mRNA in the abortion group increased after injection of exogenous IFN-γ. Density of gray-scale scanning analysis RT-PCR results showed that the expression of IL-18 in 2 IU group was similar to that of the normal pregnancy group ($p>0.05$). Expression of non-pregnancy group was significantly lower than pregnancy group ($p<0.05$). The mRNA expression level of ovarian IL-18 of abortion group, 2.4 and 2.8 IU group was extremely significantly lower than normal pregnancy group ($p<0.01$, details can be shown in Table 1, Fig. 1 and 2).

During pregnancy, expression of IL-18 mRNA in uterus was different among the test groups. The expression level of IL-18 mRNA was the lowest in abortion group. However, the expression of IL-18 had been increased after injection of exogenous IFN-γ to abortion group. Density of gray-scale scanning RT-PCR analysis showed that the expression of IL-18 in abortion group, non-pregnant group, 2.4 group and 2.8 IU group was extremely significantly lower than that of pregnancy group ($p<0.01$), the expression of IL-18 was most close to that of normal pregnant group, there was no significant difference between them ($p>0.05$, shown in Table 1, Fig. 1 and 2).

The effects of IFN-γ on the expression of IL-18 protein in ovary and uterus of pregnant rats: IL-18 protein was mainly distributed in the endometrium or decidua of tested rats. In normal pregnant rats, the IL-18 protein closely arranged and deeply stained in the endometrium or deciduas which mainly located in the nucleus (Fig. 3a). Higher expression of IL-18 could be seen in endometrial or decidual cell nuclei in 2 IU group rats (Fig. 3b), its

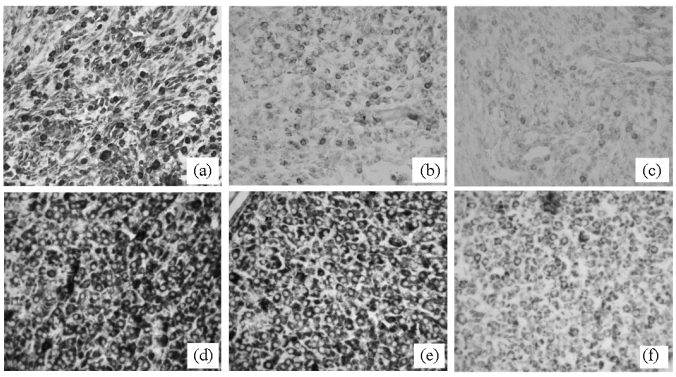

Fig. 3: The expression of IL-18 protein in the ovary and uterus of rats from each experimental group; (a) The expression in decidua of normal pregnancy group 400x; (b)The expression in decidua of 2 IU group 400x; (c) The expression in decidua of abortion group 400x; (d) The expression of IL-18 in ovarian granular luteal cells of normal pregnancy group 400x; (e) The expression of IL-18 in ovarian granular luteal cells of 2 IU group 400x; (f) The expression of IL-18 in ovarian granular luteal cells of abortion group 400x

Table 2: The expression of IL-18 protein in the ovary and uterus in the rats from each experimental groups (Mean±SD)

Groups	The relative expression in endometrium or decidua μ^2	Therelativeexpression in uterus μ^2
Normal pregnacy group	47.586±3.685	25.705±3.891
2 IU g^{-1} body weight	39.429±4.731	19.839±5.094
2.4 IU g^{-1} body weight	25.618±4.031**	15.959±4.049**
2.8 IU g^{-1} body weight	17.801±2.935**	11.081±2.907**
Non-pregnancy group	10.447±3.025**	9.642±3.421**
Abortion group	8.836±2.169**	6.864±2.976**

*Means significantly difference compared with normal pregnant group ($p<0.05$); **Means extremely significantly difference compared with normal pregnant group ($p<0.01$)

expression was close to the normal pregnancy group without significant difference between them ($p>0.05$), the expression of IL-18 protein in the endometrium or decidua in non-pregnancy group and abortion group was the lowest. The expression of IL-18 protein in 2.4 IU group and 2.8 IU group was slightly higher than abortion group but the expression of IL-18 protein in these groups were significantly lower than the normal pregnant group ($p<0.01$, Table 2, Fig. 3).

IL-18 protein positive reaction was mainly distributed in the cytoplasm of granular luteal cells with vacuolus-shaped nonstaining nucelus in ovary. The cytoplasm of granular luteal cells were deeply stained in normal pregnancy group rats (Fig. 3d). The expression level of IL-18 protein in 2 IU group was close to that in normal pregnancy group (Fig. 3e), there was no significant difference between these two groups ($p>0.05$), the expression level in abortion group and non-pregnancy group was lower. The expression level of 2.4 and 2.8 IU groups was slightly higher than the abortion group but their expression level were extremely significantly lower than the normal pregnant group ($p<0.01$, Table 2 and Fig. 3).

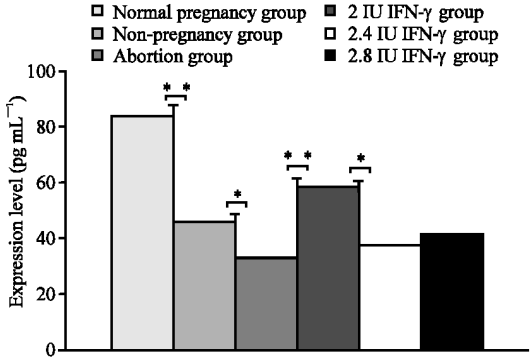

Fig. 4: IL-18 level in peripheral blood of each experimental group

The influence of IFN-γ on the expression level of IL-18 in peripheral blood of pregnant rats: The expression level of IL-18 in peripheral blood of 2 IU group rats was extremely significantly higher than that of abortion group ($p<0.01$) and significantly higher than that of non-pregnant group, 2.4 IU group and 2.8 IU group ($p<0.05$) but significantly lower than the normal pregnancy group ($p<0.05$). The expression level of IL-18 in peripheral blood of abortion rats was the lowest and no significant difference was found between 2.4 and 2.8 IU group ($p>0.05$, Fig. 4).

DISCUSSION

The establishment, maintenance of pregnancy and the start of parturition involved a variety of cytokines. It has been reported that the balance of Th1/Th2 was especially important and the state of their equilibrium decided the results of pregnancy. During normal pregnancy, the ratio of Th1/Th2 shifting towards Th2 was beneficial to the growth and development of the fetus and

their appendages. However, the start of parturition was associate with the Th1/Th2 balance (Wu *et al*., 1999). During pregnancy, there was a complex relationship of immune regulation between maternal and fetal, abortion is a form of immune rejection (Gruber and Huber, 2005). IL-18 with molecular weight of 18-19 Ku which was firstly cloned from the liver cells of the endotoxin shock rats in 1995, have many biological functions. Recently, it has been found recently that IL-18 plays an important role during the reproductive process and its abnormal expression could lead to disease. IL-18 also involvs in the regulation of Th1/Th2 balance (Cui *et al*., 2008). It has been reported that IL-12/IL-18 was involved in the regulation of uterine NK cell during development of uterine blood vessels and the polymorphism of IL-12/IL-18 gene could break the cytokine balance and increase the incidence of spontaneous abortion (Ostojic *et al*., 2007). IL-18 was also found in immune (Koibuchi *et al*., 2006; Sugama *et al*., 2007) and endocrine cells (Wang *et al*., 2006). Therefore, IL-18 was also an important immune regulatory factor in immune system and endocrine systems. It has been reported that (Gracie *et al*., 2003) IL-18 regulation on pituitary endocrine would be influenced if the IL-18 level in hypothalamus was reduced and this would affect the uterus funtion and embryo survival during the pregnancy (Gracie *et al*., 2003). IFN-γ which was mainly secreted by NK cells was one of the essential factors for pregnancy (Soares *et al*., 2007). It has been proved that IFN-γ was essential for the maintainence of the decidual cells integrity and the opening of the implantation window (Ashkar and Anne Croy, 1999).

However, overexpression of IFN-γ during pregnancy was detrimental to the pregnancy. Some studies reported that the secretion of progesterone could be inhibited by IFN-γ (Liu *et al*., 2002a, b; Thomas *et al*., 2002). IFN-γ could also induce the apoptosis of placental cells and the expression of placental antigen MHC. However, the low dose of IFN-γ was beneficial for the progress of pregnancy (Lao, 2006). In the study, after the injection of small dose of exogenous IFN-γ, the expression of both the IL-18 mRNA and its protein in ovary, uterus and the IL-18 in peripheral blood in the experimental groups were higher than the abortion group, it was speculated that exogenous IFN-γ could regulate pregnancy by regulating the expression of IL-18 in the uterus and ovaries. IL-18 had an important relationship with the establishment and maintenance of pregnancy but it may maintain embryo development in the normal course of pregnancy through the interaction with other cytokines. The effect of three doses of exogenous IFN-γ injection to IL-18 in rat uterus, ovaries were compared and the results showed that exogenous injection of 2 IU IFN-γ, IL-18 in rat uterus and ovarian during early pregnancy of the abortion group was close to the normal pregnancy group which indicating that 2 IU IFN-γ was important for the maintenance of normal pregnancy, prevention of fetal loss and early abortion.

The IL-18 in peripheral blood serum had some reference value in detecting the early abortion. Abortion was an inflammatory reaction. IL-18 was an important regulator in peripheral inflammation and host immunity response (Andrea *et al*., 2003). IL-18 could regulate the process of inflammatory catalyzed by caspase-1 at the state when inflammation happened (Fink *et al*., 2008), thereby the cell response of Th1 and Th2-type T cell was induced. The present research suggested that the levels of IL-18 in abortion rats during early pregnancy after exogenous injection of 2 IU IFN-γ was closest to the level of normal pregnancy group. It was speculated that this dose of IFN-γ could be applied in the clinical treatment of habitual abortion and infertility in humans and animals.

CONCLUSION

The results hinted that exogenous IFN-γ could regulate pregnancy via controlling the expression of IL-18 in uterus and ovaries and its level in peripheral blood during early pregnancy. Injection of exogenous IFN-γ with the dosage of 2 IU g^{-1} had an important significance for the maintenance of normal pregnancy and preventing the embryo loss and early abortion.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 39670550) and Graduate Science and Technology Innovation Fund of Northwest A and F University (No. 05ych020).

REFERENCES

Andrea, R., R.D. Wheelerb and P.D. Collinsa, 2003. Identification of a truncated IL-18Rh mRNA: A putative regulator of IL-18 expressed in rat brain. Biol. Reprod., 145: 40-45.

Ashkar, A.A. and B. Anne Croy, 1999. Interferon contributes to the normalcy of murine pregnancy. Biol. Reprod., 61: 493-502.

Boraschi, D. and C.A. Dinarello, 2006. IL-18 in autoimmunity: Review. Eur. Cytokine. Netw., 17: 224-252.

Cao, Y.Q., D.M. Sun, Y.Z. Chen and P.D. Zhu, 1999. Studies on the antifertility effect and the mechanism of action of human recombinant interferon-gamma in rabbits. J. Repro. Med., 8: 98-102.

Cui, C., S.W. Hao, B.H. Li, J.J.Hu, A.X. Zhang, J.X. Cheng and B. Shan, 2008. Study of IL218 and ICE expression in human ovarian cancer tissue. Chin. J. Imm., 24: 821-824.

Fink, S.L., T. Bergsbaken and R.T. Cookson, 2008. Anthrax Iethal tox- in and *Salmonella elicit* the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. Proc. Nat. Acad. Sci., 105: 4312-4317.

Gao, H.M. and J.S. Hong, 2008. Why neurodegenerative diseases are progressive: Uncontrolled inflammation drives disease progression. Trends Immunol., 29: 357-365.

Gracie, J.A., S.E. Robertson and I.B. McInnes, 2003. Interleukin-18. J. Leukocyte. Biol., 73: 213-224.

Gruber, C.J. and J.C. Huber, 2005. The role of dydrogesterone in recurrent (habitual) abortion. J. Steroid. Biochem. Mol. Biol., 97: 426-430.

Guan, C.N., X.H. Liao, H.Q. Luo, X.M. Chen, B.Y. Chen and P.W. Zhang, 2009. Clinical significance of serum IL-18 and IL-18BP in patients with benign or malignant primary liver tumor. Clin. Oncol. Cancer Res., 36: 365-367.

Guilbert, L., 1996. There is a bias against type 1 inflammatory cytokine expression and function in pregnancy. J. Reprod. Immunol., 32: 105-110.

Hu, C.D. and S.Q. Gui, 2004. Expression and significance of HB-EGF in trophoblast of patients with spontaneous abortion. Shanghai. Med. J., 27: 491-494.

Jin, B., W.W. Chen, B.L. Chen and H.Q. Wang, 2005. Effects of rmu- IFN-ã on the embryos of mouse infected by toxoplasma gondii in early pregnancy. Jiangsu. Med. J., 31: 206-208.

Koibuchi, N., M. Fujita, M. Hashimoto, F.S. Dhabhar and B. Conti, 2006. The adrenal gland is a source of stress-induced circulating IL-18. J. Neuroimmunol., 172: 59-65.

Lao, F.J., 2006. Effect of rmu-IFN-ã on the splenic T cell subsets and NK cells of pregnant mice infected with *Toxoplasma gondii*. Chin. J. Parasitol. Parasit. Dis., 24: 821-824.

Ledee-Bataille, N., F. Olivennes, J. Kadoch, S. Dubanchet, N. Frydman, G. Chaouat and R. Frydman, 2004. Detectable levels of interleukin-18 in uterine luminal secretions at oocyte retrieval predict failure of the embryo transfer. Human Reprod., 19: 1968-1973.

Leng, J., H. Yao, J. Shen, K. Wang, G. Zhuo and Z. Wang, 2008. Co-expression of IL-18 binding protein and IL-4 regulates Th1/Th2 cytokine response in murine collagen-induced arthritis. Acta. Biochim. Biophys., 40: 116-124.

Liu, Z., Y. Chen, Y. Yang and J.P. Peng, 2002a. The effect on MHC class II expression and apoptosis in placenta by IFNgamma administration. Contraception, 65: 177-184.

Liu, Z., Y. Yang, Y. Chen and J.P. Peng, 2002b. Influence of hr IFNγ on rabbit pregnancy. Contraception, 48: 277-280.

Ostojic, S., M. Volk, I. Medica, M. Kapovic, H. Meden-Vrtovec and B. Peterlin, 2007. Polymorphisms in the interleukin-12/18 genes and recurrent spontaneous abortion. Am. J. Reprod. Immunol., 58: 403-408.

Soares, H., H. Waechter, N. Glaichenhaus, E. Mougneau and H. Yagita *et al*., 2007. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism *in vivo*. J. Exp. Med., 204: 1095-1106.

Sugama, S., M. Fujita, M. Hashimoto and B. Conti, 2007. Stress induced morphological microglial activation in the rodent brain: Involvement of interleukin-18. Neuroscience, 146: 1388-1399.

Thomas, H.E., R. Darwiche, J.A. Corbett and T.W. Kay, 2002. Interleukin-1 plus gamma-interferon induced pancreatic beta-cell dysfunction is mediate by beta-cell nitric oxide production. Diabetes, 51: 311-316.

Tucci, M., C. Quatraro and L. Lombardi, C. Pellegrino, F. Dammacco and F. Silvestris, 2008. Glomerular accumulation of plasmacytoid dendritic cells in active lupus nephritis: Role of interleukin-18. Arthritis. Rheum., 58: 251-262.

Wang, N., S. Sugama, B. Conti, A. Teramoto and T. Shibasaki, 2006. Interleukin-18 mRNA expression in the rat pituitary gland. J. Neuroimmunol., 173: 117-125.

Wu, H., Y.P. Chang and Y.L. Yu, 1999. Th1/Th2 type cytokines in immune regulation and pregnancy. Foreign Med-Immunol., 22: 57-66.

Xie, X., H. He, M. Colonna, T. Seya, T. Takai and B.A. Croy, 2005. Pathways participating in activation of mouse uterine natural killer cells during pregnancy. Biol. Reprod., 73: 510-518.