



12/28/2015

Dear Editor,

We sincerely appreciate the positive comments from the reviewers and editors. In accordance with the requirement of the journal and the reviewer's comments, we revised the manuscript carefully. Please find the edited manuscript in Word format (file name: 14831-Review-Immunoregulation and ICP). As well, the suggested revisions are highlighted in yellow in this letter and in the manuscript.

Title: Effect of the Maternal-Fetal Interface Immunoregulation on the Occurrence of Intrahepatic Cholestasis of Pregnancy

Author: Xiu Quan Zhang, Li Juan Zhang, Wei Hong Yang, Michael L Draper

Name of Journal: *World Journal of Obstetrics and Gynecology*

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The manuscript has been improved according to the suggestions of reviewers and editors:

Comments from editors:

- Telephone number and fax number added;
- A conflict-of-interest statement is added: All authors have no conflict to this paper;
- A paragraph of Core Tip is added: **Core tip:** In this paper, we reviewed the recent publications regarding the role of immunological interactions at the maternal-fetal interface on the occurrence of intrahepatic cholestasis. The literature shows that the decidual immunological microenvironment may relate to the development of intrahepatic cholestasis of pregnancy. Any approach that modulates immune tolerance at the maternal-fetal interface toward the natural state could provide insight in the treatment of intrahepatic cholestasis of pregnancy.
- Reference numbers are changed into Arabic numbers across the manuscript. PMID and doi of the references are added.

Comments from reviewers:

- page 3" These factors can lead to vasospasm and hypoxia at the surface of the placenta and decreased vascular permeability"-I believe it is increased vascular permeability. I suggest using this reference : Sepúlveda WH1, González C, Cruz MA, Rudolph MI. Vasoconstrictive effect of bile acids on isolated human placental chorionic veins. Eur J Obstet Gynecol Reprod Biol. 1991 Dec 13;42(3):211-5.

- Responding: We appreciate the reviewer found this mistake. It is corrected as:

These factors can lead to vasospasm and hypoxia at the surface of the placenta and increased vascular permeability. The suggested reference is added.
- Page 3-" These changes can cause metabolic disorders, inadequate intake of trace elements, leading to injuries of the bile duct"-The sentences that proceeded do not lend support to this statement
 - Responding: The statement has been improved: **These changes may cause metabolism disorders. When this happens in the liver, the Na⁺-K⁺-ATP activity and the biliary bile salt transportation function may decrease, inducing intrahepatic cholestasis.**
- Page 3; "How the maternal-fetal immune influences ICP has plagued scientists"-missing word after immune
 - Responding: The sentence is improved as: **How the maternal-fetal immune interaction influences ICP has plagued scientists.**
- Page 4: " Studies have shown that Th2 type factor against Th1 type factor's immune response"; the sentence's meaning is unclear
 - Responding: We improved the sentences: **Studies have shown a shift in the balance of cytokine profiles away from Th1 type reaction to Th2 type reaction in ICP. It disrupts the immune tolerance balance between mother and fetus**
- Page 4; ref xiii does not discuss Th3. Perhaps can use reference-Prud'homme GJ1, Piccirillo CA. The inhibitory effects of transforming growth factor-beta-1 (TGF-beta1) in autoimmune diseases. J Autoimmun. 2000 Feb;14(1):23-42.
 - Responding: Agree with the reviewer's comments. The description is improved as: **Th3 secretes transforming growth factor β 1 (TGF- β 1) which has a strong immunosuppressive effect on cytotoxic T cells, natural killer cells, T cells and natural killer T cells .** The suggested reference is added.
- Page 5; BA are produced idiotypically Ab2 fetal half of this graft antigen-incomplete incomprehensible sentence. Perhaps the authors are referring to the mechanism described in this reference. This should be clarified-Reed E1, Beer AE, Hutcherson H, King DW, Suci-Foca N. The alloantibody response of pregnant women and its suppression by soluble HLA antigens and anti-idiotypic antibodies. J Reprod Immunol. 1991 Jul;20(2):115-28.
 - Responding: This sentence is rewritten as: **BA are produced idiotypically of anti-anti-HLA antibodies which are reactive with fetal half of the self HLA antigens.** The suggested reference is added.
- Page 6-typo TBF---TNF
 - Responding: Corrected.
- Page 6-The expression of TNF- α and IL-1 are decreased in placental tissue in patients with ICP, which promote the secretion of transforming growth factor 1

(TGF-1). Recent studies have found that TNF- α . It is unclear how the expression of TNF in the placenta is decreased while its level in serum is increased in ICP

- Responding: Appreciate the reviewer's point. We corrected the mistake and rewrite the section: The expression of TNF- α and IL-1 are increased in placental tissue in patients with ICP, which promote the secretion of transforming growth factor 1 (TGF-1). Recent studies have found that TNF- α increase significantly in serum of ICP patients. The increase of TNF- α is positively related to the severity of ICP, which demonstrates that TNF- α may be involved in the occurrence of ICP.
- Page 7-, leading to an increase of the Th2-Th1 ratio demonstrating-Shouldn't this be an increase in th1/th2 ratio
 - Responding: We appreciate found this mistake and corrected. These results suggest that ICP generally shows an increased Th1 type cytokine phenomenon, leading to a increase of the Th1-Th2 ratio demonstrating pathological changes of cell immune imbalance.
- Page 7; The results from these studies demonstrate that the surface antigen on Th2 cells and NK cells have enhanced cellular immune function, and promote the Th1/Th2 type cytokine balance via Th1, which may be a cause of the liver cell damage noted to be present in ICP-should mention "the lack of such balance may be a cause....."
 - Responding: Good point. We improved as suggested. The results from these studies demonstrate that the surface antigen on Th2 cells and NK cells have enhanced cellular immune function, and promote the Th1/Th2 type cytokine balance via Th1. The lack of such balance may be a cause of the liver cell damage noted to be present in ICP.
- Page 8- thophoblast-typo
 - Responding: Typewriting corrected.
- Page 8; the sentence ". The expression of HLA-II antigen compatibility is high when maternal and fetal HLA-II genes are highly similar." is redundant
 - Responding: The sentence is rewritten and improved. The compatibility is high when the mother and fetus have similar HLA-II antigen expression. The higher the compatibility of the maternal fetal HLA-II antigen, the less the immune response.
- Page 8; Nowak pointed out that women with activation of killer cell immunoglobulin-like receptor (KIR) gene and the KIR inhibitory receptor gene ratio between 0.33-0.83 were prone to have spontaneous abortion while women with a ratio between 0.86-1.25 tend to have a NK cell protective effect-Unclear. In Nowak's reference: " an excess of inhibitory KIRs (activating-to-inhibitory KIR gene ratios of 0.33 to 0.83) was associated with miscarriage, whereas ratios close to equilibrium (0.86-1.25) seemed to be protective. In addition, the results suggest for the first time that sporadic and recurrent spontaneous abortions as well as miscarriage in the

presence or absence of autoantibodies may have different KIR genotypic backgrounds"

- Responding: We agree that description is not completely clear. Nowak has a couple of studies on the immunoregulation and spontaneous abortion. The one referred to in this manuscript show that the KIR activating-to-inhibitory gene ratio is decreased in spontaneous abortion. The other reference demonstrates that among KIR AA women who have HLA-C C2C2 partners, HLA-C heterozygous females show a trend towards an increased chance of successful pregnancy. (Nowak I et al. HLA-C C1C2 heterozygosity may protect women bearing the killer immunoglobulin-like receptor AA genotype from spontaneous abortion. J Reprod Immunol. 2011 Jan;88(1):32-7. doi: 10.1016/j.jri.2010.11.001).
- Since spontaneous abortion is simple a different name for miscarriage. We agree that the description is not completely clear. However, since Nowak's study has significantly demonstrated the KIR genotype is related to spontaneous abortion. We improved the description as: **Nowak pointed out that women with activation of killer cell immunoglobulin-like receptor (KIR) gene and the KIR inhibitory receptor gene ratio between 0.33-0.83 were prone to have spontaneous abortion while women with a ratio between 0.86-1.25 tend to have a NK cell protective effect, suggesting that the KIR genotypes most likely associate with pathologic pregnancies, such as spontaneous abortion.**
- Page 8; although they explain the effect of KIR activation-inhibition; suggesting that the KIR gene types most likely determine disease susceptibility to ICP is unfounded since no reference documents such changes in ICP
 - Responding: This is basically responded in the previous comments. We added a related reference and adjusted the description.
- Page 9; Unfortunatiely; dedvelopment -typoS
 - Typewriting corrected.
- Page 9: Stress during pregnancy causes decreased IL-6 and IL-10 levels, which may be a factor related to the dedvelopment of ICP-unfounded conclusion since no studies have linked stress to ICP through such an immunologic mechanism.
 - Responding: We agree with the reviewer's comments. This section is rewritten as: **Social and psychological stress may cause an increase of proinflammatory cytokines. Stress during pregnancy causes changes of IL-6 and IL-10 levels. Even though IL-6 and IL-10 level are associated with ICP, further studies are necessary to clarify whether prenatal stress may be part of the pathogenesis of ICP.**
- Page 10- "suggesting that the regulation of expression of surface antigen on trophoblast in the ICP process can be effectively intervented"-what is meant by intervented???
 - Responding: The sentence is rewritten as: **Peng found that dexamethasone**

can treat ICP and up-regulate HLA-G and HLA-E, which suggests that the expression of surface antigen on trophoblast in ICP can be effectively influenced. This supports the notion that immune imbalance is more likely the initiating factor of ICP

- Page 10-is this "live" or "liver" cells??
 - Responding: The mistake is corrected: **liver cells**
- Page 10- mediated (at least in part) by disruption of the maternal immune-incomplete sentence
 - Responding: The sentence is completed: **Thus, these pathological changes contribute to an increased fetal morbidity due to maternal changes, mediated (at least in part) by disruption of the maternal-fetal immune balance.**
- Page 10- Because of the etiological factor and the pathogenesis of ICP, there is currently no effective way to prevent and cure ICP. Any approach that modulates the immune tolerance of the maternal-fetal interface toward the natural state would be a breakthrough in the treatment of ICP. This statement contradicts the previously mentioned role of steroids in alleviating ICP. In fact, previously the effectiveness of steroids has been used as an evidence to the immunologic basis of ICP
 - Responding: This is a good point. We appreciate the reviewer. We understand that dexamethasone has been used in experimental treatment of ICP and upregulates the expression of HLA-G on placenta. Even though we only found one study on this point, but Peng's study on dexamethasone provided what may be a significant development in treatment. Dexamethasone is clinically used to improve fetal lung maturity in babies born preterm. But it is not approved by the FDA for treatment of ICP. The purpose of this paper is to bring readers concentration to the immunoregulation of maternal-fetal interaction. The section is improved as: **Because of the etiological factor and the pathogenesis of ICP, there is currently no effective clinical standard to prevent and cure ICP. Any approach that modulates the immune tolerance of the maternal-fetal interface toward the natural state could provide insight in the understanding of ICP, which could lead to a targeted treatment.**

We thank the editors and the reviewers again for your comments and the publication of this paper to the World Journal of Obstetrics and Gynecology.

Sincerely yours,



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