Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Manuscript No 4374 Revision.doc).

Title: Fetal lung surfactant and development alterations in intrahepatic cholestasis of pregnancy

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Xiuquan Zhang

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

Reviewer 1:
    We thank for the reviewer's positive comments.

1.) Lung maturation is dependent on fetal age. The authors provide insufficient information on the baseline characteristics of the study population. Furthermore gestational age should be accounted for in all analysis presented, so that the independent association of bile acids levels on surfactant and lung maturation parameters can be evaluated. On page 5 the authors only provide the information that gestational age were from 33 weeks to 41 weeks, and that …there were no statistical differences between the ICP group and control group… for gestational age. a) The authors should provide a table comparing relevant baseline characteristics between the ICP and control group showing mean, standard deviation, median and range as appropriate together with statistical comparison between groups. b) Gestational age should be accounted for in all analysis presented, so that the independent association of bile acids levels on surfactant and lung maturation parameters can be evaluated.
The reviewer's comments on the conception is very important. We reviewed the data and added the information to compare the maternal age, gestational age. The revised paragraph is improved as following:
"There are totally 72 cases recruited in this study during 2010 and 2011. It includes 40 ICP patients  and 32 normal pregnant women with singleton pregnancy delivered using cesarean section. In ICP group, the patients age were from 18 to 40 years old and the average age were 27.7±1.37 yrs. The gestational age were from 33 weeks to $41^{+5}$ weeks and the average gestational age were 37.25±2.34 wks. In normal pregnant group, the patients age were from 19 to 36 years old and the average age were 27.2±4.67. The gestational age were from $33^{+2}$ weeks to $40^{+6}$ weeks and the average gestational age were 37.5±2.67 wks. here were no statistical differences between the ICP group and control group for maternal age, gestational age, or pregnancy times. ICP was diagnosed with the

diagnostic criteria referenced in the eighth edition of national text book of obstetrics and gynecology. Patients with liver disease, gall bladder disease, chronic vascular disease, gestational hypertension, gestational diabetes, anemia, kidney disease, heart disease, or other pregnancy complications were excluded. "

2.) The authors used ultrasonography to assess for fetal lung area. a) It is unclear when these measurements were done? Right after cesarean section? b) There are several studies on fetal lung ultrasound and measurements. It is unclear from the description, which exact method the authors used. The authors need to provide a reference for the method used and some information on operator experience, how many operators and reproducibility of the measurements. c) All measurements should be adjusted for gestational age. d) What are normal values for this measurement?

We have improved according to the reviewer's positive comments and added the reference for the description of the fetal lung area measurement. As following:

*Ultrasonography*

Color ultrasonic diagnostic system (Philips iu22, USA, probe frequency 2.5-6.0 OMHZ) were used for fetal lung area and fetal body weight within tree days to delivery. Fetal body weights were assessed and calculated by checking the fetal biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). Fetal lung areas were calculated by measuring fetal left and right lung area by freezing an image shot when the fetal heart is at the diastolic phase while the probe is parallel to the longitude line of the fetus. The area will be digitally analyzed by the computerized system automatically. Data was taken by one professional individual using mean by 3 times measurement. Total lung area and lung area/body weight will be digitally calculated. "

3.) Minor comments: There are several language and grammatical errors throughout the manuscript.

We have improved language and corrected errors in the manuscript and have native English speaker Chris Leukel from University of Utah checked and corrected.

Reviewer 2:

We are grateful that the reviewer assured that our data on surfactant phospholipid and SP-A levels and fetal lung volume in ICP are new and advances our understanding of fetal lung injury in ICP.

1. The manuscript requires language editing (construction of sentences, grammar and spelling) throughout to convey clearly the contents of the manuscript.

We have checked carefully and improved language and corrected errors in the manuscript. Furthermore, we have Chris Leukel from University of Utah, a native English speaker, checked and corrected the errors.

2. Materials and Methods section: Instead of the heading "Lab experiments", the names of methods used should be indicated. For example, ELISA, HPLC etc.

We changed the heading and briefly described the methods used in the experiments. Reference were added for readers if they are interested in the procedures of the methods. In addition, a figure with detail description of the phospholipids were presented in the manuscript. We believe all these will help the readers to understand the data. As following:

"*Amniotic fluid assay for TBA, SPA, Phospholipids:*

TBA was detected using the automatic biochemical analyzer (Hitachi 7060, Japan) with the TBA detection kit (Sigma, Shanghai Trading co LTD) following the instruction of the assay kit. The calibration was made each time using the standard calibrator. SP-A was detected with the SP-A detection kid using ELISA assay (Wuhan technology co., China and USA). Phospholipids PC, PI, LPC, and SM were detected with high-performance liquid chromatography (HPLC, Shanghai National Medicine Chemical Reagent co. LTD) with the standard phospholipids (Sigma, Shanghai Trading co. LTD). uPrasil column (300mm×4mm, 5μm) were used with a HW2000 chromatographic data station for data analysis. The procedures and steps were carried out accurately following the instructions of the agent kit and instrument. The phospholipids concentration results are show in figure 1. Amniotic fluid lamellar body was counted using hematology analyzer (ABX-Pentra120, Diomond Diagnostics, USA). "

3. Fig. 1 - I think it is unnecessary and should be deleted. Some details on extraction of phospholipids from amniotic fluid should be included.
   We believe it is more clear for readers understand our work on phospholipids measurements with the figure. Actually, with the legend explanation and the description of the methods, we hope to point out this method has constant standards for the measurement.

4. Change "Ultrasounography" to "Ultrasonography". Some details about the method used should be included. Was it 2-dimensional or 3-dimensional?
   "Ultrasounography" was changed to "Ultrasonography". A detail description was improved and a reference for the method was added.

5. Discussion section is too lengthy. It should be reduced by 50%. The statement "Phospholipase A2 plays an important role in the synthesis of alveolar surface active material" is wrong and should be deleted. References should be indicated where appropriate.
   We tried our best to simplify the discussion by deleting about one page. Also, we deleted the sentences related to phospholipase A2. References were checked and deleted ones which are not so closely related.

6. Title should be revised as follows: Fetal lung surfactant and development alterations in intrahepatic cholestasis of pregnancy.
   Answer: Changed as suggested.

Reviewer 3:
   We appreciate the reviewers comments that our experiments are well performed and en results are clearly presented.

❖ I do have some doubts concerning the SP-A results. It would be interesting to see all the measurements (eventually as supplementary information) for SP-A. This is an interesting results, so there should be no doubts about the significance. In general the manuscript is well written in good English, although some parts are written in poor English (maybe two different writers?)

We carefully checked the data and did the statistics again, for SP-A results, the probability value for the difference between the ICP and normal group is <0.05. It is significantly different. Pertaining to English, we improved and corrected carefully. In addition, we have a native English speaker checked and corrected.

Comments from Editors:

1. The revised manuscript should be in the format of Word rather than PDF.
   Format has been updated with Word Version Document.

2. For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies . If you believe that the language of your manuscript has reached or exceeded Grade A and would like to sign a guarantee. However, if we later find that the language of your manuscript has not reached Grade A, your paper will be rejected. Please highlight the changes made to the manuscript according to the peer-reviewers' comments
   This manuscript is reviewed and improved in English with the help from Christopher Leukel, who is native English speaker working at University of Utah. The changes has been highlighted.

3. Please provide a running title for this paper. no more than 6 words
   Running title provided: Fetal lung development in ICP

4. There is only one corresponding author allowed, Please make sure.
   Corrected.

5. Methods in abstract: no less than 80 words
   The methods section in abstract was rewritten and highlighted.

6. Core tip: Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers
   Core tip provided.

7. Please delete the extra space, Please check across the text.
   Extra space deleted thoroughly.

8. please put all the figures at the end of text
   All the figures are replaced in different file.

9. Please replace* by a   please  revise it in the table. so are the followings.
   Changed thoroughly.

10. Please write the COMMENTS section here. See the format in the attachment file- revision policies (Highlighted contents).
   The comments section was provided as the format.

11. Pleased provide PubMed citation numbers for the reference list, e.g. PMID and DOI,
   PMID and DOI added.

12. In addition to the improvements according to the suggestions of the reviewers, we changed, corredted, and improved some parts of the paper, which are highlighted in the manuscipts.

Thank you very much for publishing our manuscript in the *World Journal of Obstetrics and Gynecology.*

Sincerely,

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