Author's response to reviews

Title: Altered thyroid hormone profile in offspring after exposure to high estradiol environment during the first trimester of pregnancy: a cross-sectional study

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Author's response to reviews: see over
Dear Editor and Reviewers:

Thank you very much for your decision letter dated of October 17, 2014. We are glad to know that two reviewers gave our manuscript (MS Number: 7454148381401696) very positive comments. We read the comments of Editor and Reviewers carefully and revised our manuscript according to the comments and suggestions of Reviewers and Editor.

In our revised manuscript we did that: (1) we carefully revised the manuscript to address the reviewers' point by point concerns; (2) according to the reviewers’ suggestion, we reanalyzed the relationship between type of conception and thyroid function with adjustment for age of child, type of assisted reproduction, and sex of child or maternal variables by a regression model adjusting (as shown in Table 3); (3) we presented the detailed $P$ values in Table 4; (4) we reanalyzed the relationship between type of conception and thyroid function with adjustment for sex of newborns by a regression model adjusting (as shown in Table 5) according to the comments of Reviewer 2; (5) we added comments of implications of early thyroid dysfunction in later life in the discussion section; (6) we provided email addresses for all authors in the title page; (7) we revised the abstract and limited to 350 words; (8) we added the date of registration to the abstract; (9) we included all authors in the author contribution; (10) we invited a professor whose native language is English to read and edit our revised manuscript, and, corrected all spelling errors and syntax mistakes we found. On the other hand, we have 22 authors in the initial manuscript. However, we performed more analysis by a regression model according to the reviewers’ suggestion, and the data was mainly analyzed by Ye Liu. So we added Ye Liu in the author list. Now the authors is composed of Ping-Ping Lv, Ye Meng, Min Lv, Chun Feng, Ye Liu, Jing-Yi Li, Dan-Qin Yu, Yan Shen, Xiao-Lin Hu, Qian Gao, Shan Dong, Xian-Hua Lin, Gu-Feng Xu, Shen Tian, Dan Zhang, Fang-Hong Zhang, Jie-Xue Pan, Xiao-Qun Ye, Miao-E Liu, Xin-Mei Liu, Jian-Zhong Sheng, Guo-Lian Ding and He-Feng Huang. Thank you.
The followings are our responses to the comments of reviewers and editor. We hope that we answered all comments and our revised manuscript can be accepted for publication in BMC medicine. Thank you very much again for your consideration.

Best regards!

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**Response to Reviewer 1**

**Major Compulsory Revisions**

1. Lines 92-93. Why did you examine newborns conceived by fresh ET only? Any conclusion on the advantages of frozen ET cannot be drawn.

**RESPONSE:** Thank you very much for your comments. Controlled ovarian hyperstimulation (COH) leads to high serum $E_2$ level before, during and after implantation of the embryos. In our previous study, we have confirmed that $E_2$ on the day of hCG positively correlated with $E_2$ at 4 and 8 weeks of pregnancy in fresh ET group, suggesting that the serum $E_2$ level on the hCG administration day is an effective marker reflecting the $E_2$ level during early pregnancy in fresh ET group [1]. However, in frozen ET group, the embryos were transferred in the natural cycles without COH, so the serum $E_2$ level on the hCG day is at the physiological level. In other words, the serum $E_2$ level on the hCG day is not an effective marker reflecting the $E_2$ level during early pregnancy in the frozen ET group. Based on the above reasons, we examined the thyroid hormones of the newborns conceived by NC, fresh ET and frozen ET, although we performed correlation analysis of $E_2$ level on the hCG day with thyroid hormones only in fresh ET group. We are sorry that we did not explain it clearly in the article, and we have corrected it in our revised manuscript (Page 7, line 110-111). Thanks again for your comments.

2. Why is control group matched only to fresh ET? Again, then no conclusion on frozen ET cycles can be made.

**RESPONSE:** Thank you for your comments. Actually, the NC cases were matched for age and gender to cases both in fresh ET group and frozen ET group. We have corrected it in our revised manuscript (Page 8, line 127-128).

3. Selection bias? Was it a practical sample?

**RESPONSE:** Thank you for your comments. It was a cross sectional survey undertaken at the ART unit in Women’s Hospital, School of Medicine, Zhejiang University. Children conceived by IVF-ET delivered from January 2003 to March
2011 attending a routine visit at the ART unit were approached to complete a face-to-face interview. The NC cases were referred to the Physical Examination Center for regular health examination and they were recruited if they agreed to participate. Actually, we had recruited a total of 1292 Asian children (aged 3-10 yr) at the beginning, including 395 NC (389 singletons and 6 twins), 576 cases after fresh ET (364 singletons, 206 twins and 6 triplets) and 321 cases after frozen ET (216 singletons, 88 twins and 17 triplets). As is well known, the maternal intrauterine environment of twins/triplets is more complex than that of the singletons. Therefore, only singletons were included in this study. Furthermore, mothers with thyroiditis, hyperthyroidism or hypothyroidism during pregnancy were excluded (9 singletons of NC, 7 singletons of fresh ET and 4 singletons of frozen ET were excluded). Finally, 949 singletons remained in this study, including 380 NC, 357 cases after fresh ET, and 212 cases after frozen ET. Besides, in order to minimize selection bias in our study, all cases were randomly selected, examined by a trained female nurse, and more than 3 researchers evaluated the results. Thanks again for your comments.

4. Mothers with thyroid disease during pregnancy were excluded. Please define. According to existing guidelines the TSH threshold for first trimester is 2.5 \( \mu \text{U/ml} \). Did women with TSH ranging from 2.6 to 4.5 included as well? (They would have not been diagnosed at that time).

**RESPONSE**: Thank you for your comments. In this study, mother with typical symptoms of thyroiditis, hyperthyroidism or hypothyroidism during pregnancy were excluded. We did not explain it clearly in the article, and we have corrected it in our revised manuscript (Page 8, line 132-133). As you said, guidelines of the American Thyroid Association (ATA) proposed that the upper limit of the TSH reference range should be 2.5 mIU/L in first trimester. However, the reported ranges in China are significantly higher [2]. According to this, in our study, we did not exclude the women had not been diagnosed at that time.

5. The children have different ages and TSH is influenced by age in children. Unless
you include children of the same age, no comparisons can be made.

RESPONSE: Thank you for your good comments. We fully agree with you that TSH is influenced by age in children. In order to solidify the results, we reanalyzed the relationships between type of conception and thyroid function with adjustment for age of child, type of assisted reproduction, and sex of child or maternal variables by a regression model adjusting. We have added the results to Table 3 in our revised manuscript.

6. Absolute differences are really small among the three groups when it comes to TSH concentrations.

RESPONSE: Thank you for your comments. Indeed, the absolute differences were small among the three groups when it comes to TSH concentrations. Therefore, we further compared the incidences of 3-10yr children whose serum level of T4, FT4 or TSH beyond the standard values among the three groups. Interestingly, we found that the incidence in fresh ET group was significantly higher than those in NC and frozen ET groups. In fresh ET group, some children showed elevated T4 and/or FT4, and some showed elevated TSH alone, although most others were normal. Sakka et al. studied 106 children conceived after classic IVF and 68 naturally conceived controls, aged 4-14yr, and found that seven IVF children but none of the controls had persistent elevations of circulating TSH. They also found that the level of TSH was significantly higher in the IVF group than in controls, which is consistent with our results. Also, their absolute difference of TSH between IVF and control group was small [3]. Perhaps, the sensitivity to high E₂ environment was varied among individuals. Therefore, although the absolute differences were small, the result is statistically significant and has clinical significance.

Minor revisions

1. Intrauterine estradiol. This does not seem to be the case. The authors use the term "intrauterine estradiol" throughout the manuscript, whereas they only measure estradiol in serum.
RESPONSE: Thank you for your comments. What we want to emphasize is that in fresh ET group, when the fetus is in the uterine, the serum estradiol level of the mother is much higher than normal physiological level. Since we could not detect the estradiol level in the uterus, we adopted the estradiol level in serum. The term “intrauterine estradiol” may be not accurate, so we replaced it with “maternal estradiol”. We have corrected it in our revised manuscript.

2. Lines 53-54. Please provide references.
RESPONSE: We have provided the references in our revised manuscript (Page 5, line 64-65). Thank you for your suggestion.

3. Lines 64-65. Early screening for thyroid function is already a norm in most countries.
RESPONSE: Thank you for your comments. We have deleted the sentence in our revised manuscript (Page 5, line 78-79).

4. Lines 78-83. There is no need to explain basic thyroid physiology.
RESPONSE: Thank you for your suggestion. We deleted the explanation of basic thyroid physiology in our revised manuscript (Page 6, line 94-99).

5. Lines 84-85. Please provide references.
RESPONSE: We have provided the references in our revised manuscript (Page 6, line 101-102). Thank you for your suggestion.

Reference

Response to Reviewer 2

1. In the first study of children age 3-10, the authors report relationships between type of conception and thyroid function without adjustment for age of child, type of assisted reproduction, sex of child or maternal variables. A regression model adjusting for these variables would help to solidify the results.

RESPONSE: Thank you very much for your excellent comments and suggestion. In order to solidify the results, we reanalyzed the relationship between type of conception and thyroid function with adjustment for age of child, type of assisted reproduction, and sex of child or maternal variables by a regression model adjusting. We have added the results to Table 3 in our revised manuscript.

2. A similar analysis could be done for the newborn study.

RESPONSE: All newborns included in our study were free of obstetric complications during pregnancy, and, conceived after traditional IVF. Therefore, we reanalyzed the relationship between type of conception and thyroid function with adjustment for sex of newborns by a regression model adjusting. We added the results to Table 5 as you suggested. Thank you.

3. The authors do not report the numbers of children with the various combinations of abnormal thyroid results. It would be interesting to see whether children with one abnormal parameter also had other abnormal parameters. In fact, the outcome could then be binary and a logistic regression model could be developed to assess the relationships while controlling for other possible confounding variables. This could be done for both the older child study and the newborn study.

RESPONSE: Thank you very much for your comments and suggestion. Actually, we only found one child showed both elevated FT4 (> 19.05 nmol/L) and TSH (> 5.05 mIU/L) in fresh ET group, whereas none in frozen ET and NC group. There was no significant difference among three groups. None of the newborns showed two abnormal parameters at the same time. Therefore, we did not perform the logistic
regression model to assess the relationships while controlling for other possible confounding variables in Table 4. The detailed $P$ values were shown in Table 4.

4. A discussion of the implications of early thyroid dysfunction in later life would add to the manuscript, i.e. what does early dysfunction mean for behavioral, cognitive and metabolic disorders later in life.

**RESPONSE:** Thank you very much for your suggestion. We have added a discussion of implications of early thyroid dysfunction in later life in the discussion section (Page 19-20, line 340-356). Thyroid hormones play an important role in regulating lipid metabolism, and thyroid dysfunction can result in lipid abnormalities which increase the risk of hypertension, cardiovascular disease and endothelial dysfunction [1]. The increased cardiovascular risk in thyroid dysfunction is related to lipid profile, endothelial dysfunction, metabolic, hormonal and hemodynamic changes and coagulation disturbances [2]. Caraccio et al. reported that subclinical hypothyroidism adversely affects some surrogate markers for cardiovascular disease [3]. Moreover, previous study reported that mental changes were always accompanied with thyroid dysfunction, perhaps due to the stimulation by the protean actions of thyroid hormone, and thyroid gland might play a contributing part in the pathogenesis of the psychiatric disorders [4]. Accumulating studies showed that IVF children had shown significant increases of arterial blood pressure and adipose tissue distribution as well as a higher level of fasting blood glucose [5-6]. In addition, recent studies in IVF conceived mice have displayed increased fasting glucose and impaired glucose tolerance [7]. These cardiometabolic alterations and lipid metabolic disorder in IVF children might be partly attributed to a higher occurrence of thyroid dysfunction. Therefore, early occurrence of thyroid dysfunction, if not promptly treated, will cause serious and irreversible damage to the cardiovascular system, and predisposes to dyslipidemia, mental changes and metabolic disorders later in life.

**Reference**


