Author’s response to reviews

Title: The effects of daily meteorological perturbation on pregnancy outcome: Follow-up of a cohort of young women undergoing IVF treatment

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Author’s response to reviews:

Dear Editor,

Thank you for arranging a timely review of our manuscript. We are pleased to know that our work is of general interest for the readers of Environmental Health. We have carefully evaluated the reviewers’ comments and thoughtful suggestions responded to these suggestions point-by-point and revised the manuscript accordingly. All changes made to the text are in tracked changes and with annotation corresponded to the specific point so that they may be easily identified. With regard to the reviewers’ comments and suggestions, we wish to reply as follows:

RESPONSE TO REVIEWER #1’s COMMENTS:

1. Line 50-51: The authors stated that "assisted reproduction provides a good model for investigating the sole meteorological effects in reproduction as most variables are controlled". In what way are the variables controlled? I think the authors should more precisely say that "… the periconceptional periods and the meteorological variable around those periods were easily and precisely traceable".
Response: Thank you for your suggestion. We have now revised the sentence as followed “Assisted reproduction provides a good model for investigating the sole effects of meteorological changes on reproduction as the periconceptional periods, patient’s physiological status, and the meteorological variables during the process of assisted reproduction can be easily and precisely determined” (line 68-71)
2. Line 77: why was ethics approval not required. This study involved analysis on patient data and hence ethics approval should be sought. Did the authors actually mean that "written consents from the subjects" were not required? This may be reasonable for retrospective studies involving only clinical record reviews if this was endorsed by the ethics committee as such.
Response: Thank you for your comment. We apologized for the misleading sentence. We have now applied for ethics approval for this retrospective analysis and will be available shortly. We revised the sentence in as followed “The retrospective study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee, and the requirement for obtaining written informed consent from the patients was waived.” (line 110-113)

3. Line 82: On what basis did the authors consider that the effect of meteorological variations can be shown only in patients treated on the long protocol but not the antagonist protocol? The period of ovarian stimulation in women treated on the antagonist protocol is still considerable to allow the effects from meteorological variations to be manifested. In the analysis, the authors were counting in the meteorological parameters during the ovarian stimulation only, and so what happened in the pre-stimulation period were not as relevant anyway. Furthermore, antagonist protocol is a mainstay protocol in recent years, and it would be more meaningful to include women treated on the antagonist protocol.
Response: Thank you for arising this point. We do agree that the period of ovarian stimulation in women treated on the antagonist protocol may allow the effects from meteorological variations to be manifested. The scope of this study is only focusing on the meteorological variations from the day of stimulation applied. Moreover, after analysing the patient treated with the long protocol, we also analysed the patient treated under other protocols and found no significant results and the data were supplemented in the revised manuscript. In the revised manuscript we have revised the sentence in the discussion “Compared to other short-term protocols (data shown in Supplement 2, non-significant), the significance of the long-term protocol indicated that it takes a longer period of time for the effects of temperature on the pregnancy outcome to become apparent.” (line 398-401)

4. Line 88: "Patients with missing/wrong CYCL, OR, ER records or pregnancy outcomes were excluded". Did the authors mean that data on the CYCL, OR and ER dates were missing? I would think that these essential parameters should be reasonably easy to trace back from clinical records (either hard copy or electronic) of patients kept in any ART unit. Also, how would the authors know that some data were "wrong"?
Response: Thank you very much for your comment. We first find the missing data in the electronic system and found they were recorded ten years ago. Since the electronic system upgraded for several times, and some of them was not able to trace back. Nevertheless, we have found them back through the hard copy. As respect to the “wrong” data, it means the obvious wrong records i.e. “the patient first treated in 2007, but her ER was in 2005”. We have corrected them via the hard copy. Therefore, this sentence was revised as followed “We traced the original medical records in patients with missing or incorrect electronic IVF records (i.e. first treated in 2007, but embryo transferred in 2005).” (line 125-128)

5. It appears to me that some patients were included in this study for more than once. It would be the wrong approach. Only the first treatment cycle of each subject should be included to avoid bias. Otherwise, subjects undergoing repeated treatment (who generally are those with poorer prognosis) would be over-represented.
Response: Thank you very much for pointing this. On reflection, we do agree if we recruited patients...
treated more than once it would induce bias. Per your suggestion, we now divided our patient pool into three parts (the first part was discussed here, and the second part was discussed in point 6 and 7, and the third part was epidemics analysis required by the reviewer 2). In the first part, we selected all the first cycle to analyse and re-analysed the data in our revised manuscript. The results still support the original conclusion.

We added a paragraph in the revised manuscript “The cohort was divided into the following three groups: Group I included all eligible patients who underwent the first IVF cycle; Group II included patients with two cycles who had one successful and one failed pregnancy outcome as a case-control cohort for the intra-patient subgroup analysis; and Group III included all patients neglecting their number of treatment or cycle, and grouped their successful pregnancy events and variables in a monthly level to conduct an epidemic time-series study.”

We revised a paragraph in Intra-patient subgroup analysis section “To further confirm the meteorological effects and seasonal pattern showing significant effects on pregnancy outcome, the patients who underwent one successful cycle and one failed cycle were categorized into the part II cohort as a case-control cohort to eliminate all of the inter-patient variations as they were treated under the same protocol (long protocol).”

We also revised the results part “The results of the intra-patient subgroup analysis are consistent with the one in the original population. Among our 1029 cycles, there were 43 patients who underwent two cycles with only one clinical pregnancy outcome. Demographic data of patients and laboratory outcomes are listed in Table 4. The proportion of this subgroup of patients was balanced in both clinical and laboratory data. The analysis of meteorological variation (Figure 3) shows, that in this subgroup of patients, the pregnancy probability increased during CYCL to OR and OR to ER. Table 5 shows the unadjusted and adjusted OR and CI range of different meteorological variations. The multivariate analysis shows the mean temperature from CYCL to OR and OR to ER have a significant association with pregnancy probability (aOR = 1.12 and 1.14, respectively).”

6. Hence, I think the "intra-patient subgroup analysis" described in lines 209-224 should be deleted, and it is not the correct approach to perform the analysis.

7. On the other hand, if the authors would like to study the "intra-patient" variations, the repeated measures approach can be used for statistical analyses instead, comparing the first and second cycles (equal number of cycles should be compared within each subject). The absolute change in the meteorological parameters may be correlated with the pregnancy outcome. However, the interval between the two cycles should be adjusted for. A statistician may be consulted for the best approach to perform such analysis.

Response: Thank you for your useful advice. We conducted the “intra-patient subgroup analysis” for the purpose of verifying the meteorological impact on clinical pregnancy from a different angle. Per your suggestion, we now divided our patient pool into two parts (the first part was discussed in point 5). In the second part, our statistician suggested us to perform the “intra-patient subgroup analysis” with patients who had one successful and one failure pregnancy results, which represents a case-control study. This can balance the variable in individual levels using a repeated measured structure design. For the modelling, we used a mixed-effect model to control the repeated measured structure of data. For each patient, we specified an intercept for each patient, thus, the measurement of other fixed effects like season and weather variables in an individual was sharing the same intercept.

8. Please describe the luteal phase support protocol in the methodology.

Response: Thank you for your comment. In the revised manuscript we added the following description on the luteal phase support protocol “Luteal phase support was provided either by
progesterone administration or human chorionic gonadotropin (hCG) intramuscular injection from the day of OR. Luteal support was provided till 8–10 weeks of gestation.” (line 153-156)

9. Lines 128-130: for the meteorological parameters of each woman, was the mean over the CYCL-OR and OR-ER period being taken for analysis? Please state.
Response: Thank you for giving us the opportunity to clarify this sentence. We have added more information on the revised manuscript “These variations were matched with the duration of patients’ treatment daily — from the first day of CYCL to the day of ER, and the mean value of the matched variation over the CYCL-OR and OR-ER period was taken for analysis.” (line 172-174)

10. Lines 151-152: were the continuous variables normally distributed? If not, they should be presented as median (25th - 75th percentiles) and be compared by non-parametric test (Kruskal-Wallis test) instead of parametric ANOVA.
Response: Thank you for your kindly comment. We have revised this point in the manuscript in the Statistical analysis section as well as Table 1 and Table 4.

11. Lines 159-160: please spell ID in full.
Response: Thank you for pointing out this. We have spelt the ID in full and examined the correctness of abbreviation used in the revised manuscript.

12. Lines 169: why were the records missing? Could they be traced from the clinical record (either hardcopy or electronic), which I think all ART units should be keeping.
Response: Thank you for your comment. Per your suggestion, we have traced the record by checking their original medical record.

13. The authors analysed the CYCL-OR and OR-ER periods separately, which makes sense as the former may be more relevant for follicle and oocyte development, which the latter may be more relevant for the uterine environment (since the gametes and embryos were detached from the women during this period) if such effects exist. However, I do not think the CYCL-ER period needs to be reported as it just complicates the picture.
Response: Thank you for your valuable comment. On reflection, we do agree with the analysis of CYCL-ER when the gametes were apart from the patient was of less relevance to the topic here and complicated the picture. We have deleted it in the revised manuscript.

RESPONSE TO REVIEWER #2’s COMMENTS:

1. please review on using DNLM for analysing associations between weather variables and health outcomes. This approach takes into account the non-linear associations.
Response: Thank you very much for providing such a statistic to us to further analyse the weather variables and pregnancy outcome. We have used DNLM to analyse our data and presented as followed in the revised manuscript.
We added the followed paragraphs in the revised manuscript,
Method section:
“Time-series analysis
To further analyze the confirmed meteorological effects on pregnancy outcome at the epidemics level, we grouped all variables and successful pregnancy events in a month level and use distributed lag linear and non-linear models (DLNMs) to analyze their association.” (line 196-199)
“Besides, for meteorological effects showing significance, we analyzed their time-series trends in the epidemic level. To do this, the numeric variables were summarized to the month level by calculating the mean of each patient in a specific month, and categorical variables, such as fertilization method, were summarized as proportions. The DLNMs were used to evaluate the relationship between a meteorology variable and the number of successful pregnancy events separately [20]. Each model assumed an overly dispersed Poisson distribution of pregnancy events outcome and included a meteorology variable and covariates (mean age, year of the cycle, gonadotropin dose, transferred embryo grade, and proportion of fertilization method). We modelled the effect and lagged effect of meteorology variables through a natural cubic spline with 3 degrees of freedom and specified the lagged effect up to 6 months. The DLNM package was used to perform the modelling [21].” (line 226-236)

Result section:
“Epidemic time-series analysis
The data of the original population was summarized with a monthly time-series format to explore the association between mean temperature and pregnancy events. The relationship between the effect of temperature and pregnancy events is shown in Figure 6. A general positive association can be observed between temperature and the relative risk (RR) from 12°C to 25°C. A decreasing trend with fluctuation is then noted in different lags. The prediction of RR for different temperatures is shown in Table 6. With 21.5°C as the reference temperature, the RR and its CI for each unit increase from 12°C to 25°C was calculated. The CI indicates that only the positive association between 12°C and 16°C was significant, and an increasing and decreasing trend is noted for the RR, with a turning point at 25°C, which is similar to that shown in Figure 6.” (line 330-340)

Discuss section:
“When we review the association in a time-series pattern and epidemic level, the analysis by the DNLM method provided another viewpoint for supporting the abovementioned findings. In lag 0–1, that is the month of treatment, the DNLM result shows trends consistent with the results of our regression analysis. Moreover, we found a significant effect—acute and long-term negative effect—of low temperature (&lt;16°C) on pregnancy, which partially accounted for the relationship between temperature and pregnancy. However, although the trend of RR is clear, their CI for temperatures &gt;16 °C has become non-significant. Thus, from the epidemic aspect, the relationship between temperature and pregnancy still need to be verified by further studies.” (line 368-376)

2. Also, the methods section needs to be written more clearly so that other scientists could replicate the analyses.
Response: Thank you for your comment. We have re-written the method section. We have described the building of our analysis model in the Statistical analysis section in detail. We hope this version will be clearer for other scientists to replicate the analyses.

3. Finally, the manuscript needs to be edited by a native English speaker.
Response: Per your suggestion, we have asked a native English speaker to polish our paper. We have provided the certificate of editing in the Supplementary Material 3.