Author's response to reviews

Title: Do intrauterine or genetic influences explain the foetal origins of chronic disease? A novel experimental method for disentangling effects.

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Author's response to reviews: see over
Reviewer’s reports
Dawn Misra

1. The importance of gene-environment interaction.
We have now mentioned gene-environment interaction and cited the recent Institute of Medicine report. We have referred interested readers to key references but to keep this paper focused on the issue of mediation of prenatal risk effects, we have not attempted to provide a comprehensive overview on genetics. We have highlighted that the focus of this design comes about because of gene-environment interplay i.e. the non-independence of exposure to environmental risk factors and genetic liability.

2. The maternal and foetal genome are correlated.
The design rests on this and we have expanded our discussion to clarify this and also added a figure to better explain this.

Genetic influences on risk factor and outcome e.g. smoking and ADHD.
We have considerably expanded what we have written on this and used more examples to clarify what we mean. We have deleted the example on smoking and ADHD. We have also included animal study cross fostering designs to try and better explain what we mean.

Further explanation using the different scenarios.
We have expanded the discussion on this throughout the paper and used animal studies to further illustrate. We have also now mentioned that the sample could be used to investigate contribution of genes and environment but not greatly expanded this as that is not the real attraction of this design. Twin and adoption study designs already allow this.

Smoking and ADHD.
We have provided alternative examinations to better clarify what we mean.

Different scenarios for different groups
We have provided an example (gestational stress and offspring anxiety) to illustrate a potential scenario as described in table 2.

3) Data from surrogates
The reviewer is correct in stating that pregnancy data from gestational surrogates is not available. We had written this in the last section of the paper. However we have now clarified that the design does not rest on the surrogacy group alone, it relies on mothers carrying genetically unrelated offspring. It is for this reason we amalgamated groups in the way that we did in table 2 as that is how we plan to group the samples for the primary analyses of testing for environmental mediation. However in view of the reviewer’s comments we have added in further detail on this and provided data for each of the groups separately.
4) **Imprinting effects, mitochondrial DNA**
We have mentioned epigenetics as it is relevant to the issue of non inherited mechanisms accounting for prenatal environmental effects but have not attempted to discuss genetics in greater detail (including mitochondrial DNA and other genetic effects) as that is not relevant to the key aspect of this design and for complex disorders traditional human genetic epidemiological designs can not tease out specific biological mechanisms.

5) **Suitability for BMC Medical Research Methodology.**
We would be pleased for the paper to be considered by BMC Medical Research Methodology, if felt appropriate by the Editor.
Richard Ijzerman

1. **More suitable as methodology paper**
   As stated before, we are happy for the paper to be considered for a methodology section.

2. Pre-implantation environment
   We agree this is important and have highlighted that this is a consideration for the future. In Table 3 we provide information on the different groups now.

3. Dutch famine and cardiovascular risk factors
   These have now been mentioned and cited.
Anastasia Iliadou

General
Further description needed.
We have expanded the paper and increased detail on description and added in an additional table and a figure.

Major comments
1. Table 2 rather confusing
We agree that this might not be clear and have clarified in text that we are here focusing on the woman undergoing the pregnancy and that where there is both genetic and environmental mediation, we would see an association in all groups but an increased association in genetically related groups. We have provided an example (gestational stress and offspring anxiety). We have also clarified on table 2 that we are referring to the genetic relationship between the woman undergoing the pregnancy, that is not the “social” mother and the offspring. We have also provided an example of what to expect if both genetic and environmental mediation were contributing.

If there is genetic mediation between birth weight and risk of ADHD, would we not expect to see association in the gestational surrogacy and egg donation group?
We have used gestational stress as this is probably a better example. If there was genetic mediation between prenatal stress and offspring anxiety, there would be association in the groups where the woman undergoing the pregnancy is genetically related to the baby. That is not the case for gestational surrogacy and egg donation. We have expanded our discussion and given examples of prenatal cross fostering studies in the animal literature that hopefully clarifies this.

Table 2
There are two different types of questions that can be answered. Investigating the contribution of genetic and environmental contribution is interesting so we have now mentioned this in the revised manuscript. However it is important that this was not the main aim of this design. The novel aspect of this design is essentially to undertake a human adoption study “in utero”. We have now discussed this and hope it is now clearer. Paternal genes, within this context, in so much as we are looking at maternally provided environment and the effects that might be contingent upon maternal characteristics, are not important. We have highlighted that it is maternal provision we are dealing with throughout the paper and used animal examples.

2) Distinguishing G and E.
As mentioned above, the focus of this design is on the prenatal environmental mediation test but have now mentioned the other use of the design –examining genetic contribution and environmental influences. We have pointed out that other designs can be used to examine genetic and environmental contributions to
phenotypic variation but not for testing whether maternally provided prenatal environmental effects are environmentally mediated.

3) Numbers in the Results section and Table 1
These have been updated and the numbers corrected. We have clarified that our expected sample size is based on a) power calculations and b) likely recruitment based on number of types of IVF treatments in the UK for the given age range and pilot data.

4) Child characteristics
We have added further information on child characteristics in the text, cited previous published work and provided the numbers of twins. We have not completed sample collection and thus the aim here in this paper is to highlight the design and the issues that need to be considered.

5) Twin study designs
We have mentioned twin study designs. Twin and adoption study designs are excellent methods (methods we have used ourselves for more than a decade) but do not allow testing whether prenatal (not postnatal) environmental risk effects are environmentally mediated. Discussion has been expanded to clarify this.

6) Generalisability
We have cited literature and highlighted that this is a potential limitation. We have also mentioned though that for testing environmental mediation it is the representativeness of association between predictor and outcome rather than the mean values that need to be considered. We have added in the effect sizes we are able to detect based on initial power calculations (following a pilot study).

Minor comments
1. “natural experiment” in abstract.. We have removed this
2. Results section –we have provided the numbers with frequencies in brackets
3. We have corrected the references
1. **Discussion of types and rates of disorder.**
   This has now been added. We have mentioned that it is common disorders and traits that can be usefully studied.

2. **Importance of follow-up**
   We agree and have added this in.

3. **Equivalence of data on IVF children**
   We have cited literature and added information on the children in text.

4. **An explicit discussion of how the data will be analysed.**
   We have explained how data will be analysed which is exactly as this reviewer says. We have reworked discussion of Table 2 and hope it is clearer now.

5. **Power estimates**
   The expected numbers were based on power calculations and on what was feasible. The power calculations have been included and as this reviewer says there is power to detect modest effect sizes even if the surrogacy group is small.

6. **Numbers of pregnancies resulting in multiple births**
   This has now been added.

7. **True useable sample sizes**
   We have now included information on this. Where the focus is on prenatal variables, maternal reports alone are satisfactory in most instances. Thus the eligible sample here will include all those families where the mother has returned a questionnaire.

8. **Level of agreement between maternal report and antenatal notes**
   We have provided numbers.

9. We have corrected the reference error.

10. **Is there an error in Table 1?**
    We have explained we have over-recruited homologous IVF families.

11. We have changed Table 3.

12. We agree the Table 2 column is misleading. We have corrected this, changed the text and hope this has clarified the paper.