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Reply: To pool or not to pool DNA methylation data from different tissues?

Sir,
We would like to thank Dr van Montfoort for their letter highlighting a number of important issues related to our article (Lazaraviciute et al., 2014). In relation to the inclusion of different tissue types, in our manuscript we made the same points as Dr van Montfoort and emphasized this in our recommendations for future studies in this field. We stated that: ‘Where possible it is useful to also sample DNA from a second tissue (most likely blood) to confirm the soma-wide generalizability of the findings’. Specifically in relation to placental DNA, we recommended that ‘Interpretation of placental methylation data is complicated; therefore it is better to avoid this tissue when the aim is to investigate methylation changes in the offspring’.

The decision to pool the data across tissue types for this meta-analysis was not solely based on the study by Byun et al. (2009). We also noted that ‘Many imprinted genes maintain their allele-specific methylation signal in a wide range of adult human somatic tissues over decades (Sandovici et al., 2003; Coolen et al., 2011; Woodfine et al., 2011)’. But the considerations here are complicated. Some imprints may differ between cell types but, even for those that do, if they rank in the same way in individuals then the generalizability of the findings in one tissue may be valid. Even universality across tissues is not necessary for the issue of whether ART can influence imprints in a way that may impact on offspring health.

In terms of the regions measured we agree with Dr van Montfoort that methylation in different regions within an imprinted gene can have different functional effects and that a lack of difference in one region in response to assisted reproduction does not preclude a functionally important effect in some other region. In the Conclusion section we stated that: ‘Heterogeneity in the types of fertility treatment, the imprinted regions studied, the tissues used and the methods of measurement, reduce our ability to assess the full effect of ART on DNA methylation and imprinting.’ It is of course in the nature of meta-analysis that data are aggregated and we stated explicitly that ‘We used a liberal approach in terms of aggregation of methylation data from different types of tissue samples although there is a case for considering placental imprinting methylation separately.’ We decided to include as much information as we could whilst clearly labelling the data so that readers can assess and interpret the quality of the evidence in the context of their own views on this subject.

Our aim in conducting the meta-analysis was to both summarize the information available so far and to try to ensure that future study designs are as useful as possible when attempting to evaluate the effect of ART on imprinting; a topic potentially important to the safety of this technology. In the Conclusion section, our recommendation for future studies, i.e. ‘More controlled studies, using standardized methodologies, in larger, better clinically defined populations are needed’, is entirely consistent with Dr van Montfoort’s view.

Our supplementary table 3 includes methylation data for H19 from both Nelissen et al. (2013) and Puumala et al. (2012) by specific regions—CTCF 3 and CTCF 6—as in the original papers. However, we were unable to aggregate these data with those from other papers that were included in the H19 meta-analysis which analysed the region as a whole. We encountered the same situation for PEG1/MEST. Nelissen et al. (2013) reported data for alpha and beta regions of PEG1/MEST rather than the whole region, making it impossible to include the results in the meta-analysis.

As stated in the methods section, our decision to use either a fixed or a random effects model for the actual meta-analysis was based on statistical heterogeneity as indicated by the magnitude of the $I^2$ statistic. In the KvDMR meta-analysis, a fixed effects model was used as the $I^2$ was < 50%.

We are grateful to Dr Montfoort for pointing out a number of discrepancies within Figure 3 and have taken this opportunity to correct these by rechecking our data and generating an amended version (Lazaraviciute et al., 2015).

We acknowledge the inconsistency between data in the meta-analyses (Figure 3) and those presented in supplementary table 2 (published results from individual studies). This is partly due to the fact that we obtained additional data from authors of included studies over and above what was available in their published papers. This is stated in our methods section and we would like to thank all the authors who sent additional data on our request. The published data from original papers which are in the public domain have been reported in the supplementary tables, whereas data based on correspondence with both Drs Rancourt and Puumala, who provided more specific details were used for the meta-analysis.

We have corrected all identified errors in Figure 3 the Journal has published a Corrigendum (Lazaraviciute et al., 2015). It is worth noting that these adjustments have not resulted in any change in the overall findings or the conclusions of this review.

References
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