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

Sir,

With great interest I read the paper of Lazaraviciute et al. (2014) in your journal. In this review, the authors show the results of a meta-analysis on imprinted gene DNA methylation effects of IVF/ICSI on the forthcoming children. With numerous studies focussing on health of IVF children and the potential risk of adverse developmental programming, this meta-analysis as such is very timely and highly needed. I would, however, like to discuss the way in which the analysis was performed.

The studies included in the meta-analysis are, as the authors also state in their discussion, very heterogeneous regarding the assisted reproductive technology (ART) methods used, the population included and the tissue that was analysed. This makes their attempt to perform a meta-analysis quite challenging. I would like to express my hesitations with aggregating the data obtained from different cells and tissues. The samples analysed include placenta, peripheral blood, buccal smear cells and cord blood, or in other words, tissues derived from both the embryonic and extra-embryonic line. Although the authors briefly mention that there may be differences in the epigenetic status of these tissues, I think that this point is too easily neglected. The decision to pool the data on different tissues was based on a study by Byun et al. (2009). However, in this study neither placental tissue nor blood was analysed and no specific attention to the germline differentially methylated regions (DMRs) of imprinted genes was given. Studies that did focus on specific DMRs in placental tissue and blood or buccal cells found differences, i.e. hypomethylation of IGF2-DMR2 and H19 promoter DMR in placenta compared with peripheral and umbilical cord blood (Tabano et al., 2010). The tissue specific DNA methylation is also visible in Figure 3D of the review, where the level of IGF2 methylation in the study of Puumala et al. (2012) is higher in lymphocytes than in buccal cells. Such differences are not necessarily a problem for a meta-analysis when it is assumed that the effect of IVF/ICSI on the DNA methylation is similar in all tissues. The question is whether this is true. Animal studies on this topic have shown differential effects of IVF on embryonic and extra-embryonic tissue. Fauque et al. (2010) showed for instance in mice that at E10.5 the methylation level at IGF2 and H19 DMR is lower in placental tissue than in fetal tissue, and that IVF and embryo culture lead to hypomethylation of H19 DMR in the placenta, but not in the fetus. Similar results are described by Mann et al. (2004); loss of methylation at H19 DMR and Smrprp promoter in placenta tissue but not, or to a lesser extent, in fetal tissue. In my opinion it is therefore questionable whether it is correct to pool the data as done in the meta-analysis, as it might hide tissue specific IVF/ICSI effects in the human and hence will prevent the acquisition of knowledge on this topic beforehand.

Another issue that might affect the interpretation of a meta-analysis on this topic is the region within a DMR that is analysed. As we have shown, within the H19 DMR the CTCF binding region 3 was not affected by IVF/ICSI, while the CTCF binding region 6, which is known to regulate gene expression, was hypomethylated in the IVF/ICSI group (Nelissen et al., 2013). Interestingly, these data and the data from Puumala et al., about the two H19 DMR regions, as well as the data on the promoter regions of both MEST transcripts that we analysed were not included in the meta-analysis. The question is why not, and whether the authors took into account the specific region within the DMRs that was analysed in the studies.

Finally, I have some questions about Figure 3 of Lazaraviciute et al. (2014). It is highlighted that part of the data from the Puumala study are from placenta tissue. Puumala et al. (2012) did not analyse placenta, only lymphocytes and buccal cells. It struck me that the standard deviations in the study of Oliver et al. (2012) were small compared with the other studies, and comparison with the original Oliver et al. paper showed that these are the SEM instead. Why was a fixed effect model used for the analysis of KvDMR and a random model for the other genes? It is unclear where some data come from as these are inconsistent with the data presented in supplementary table 2 of Lazaraviciute et al. (2014); the results from individual studies. These inconsistencies concern the number of included subjects in the IVF/ICSI and control group in the Rancourt study and the mean DNA methylation values and the number of subjects in the Puumala study, especially since the number of subjects is sometimes higher than in the original publication.

References


Puumala SE, Nelson HH, Ross JA, Nguyen RH, Damario MA, Spector LG. Similar DNA methylation levels in specific imprinting control regions in children conceived with

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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 I² statistic. In the KvDMR meta-analysis, a fixed effects model was used as the I² 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