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NEWS AND COMMENTARY

**Imprinting, Small Babies and Assisted Reproduction**

# Genomic imprinting, small babies and assisted reproduction

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On page 22 of this issue, Kanber *et al*[1] seek evidence that abnormal genomic imprinting is a cause of reduced intrauterine growth in children conceived using assisted reproductive technologies (ART). Although Kanber *et al*[1] find little evidence to support this hypothesis, their research brings together two important fields of research regarding the health of children conceived using ART.

Over the last 5 years it has become clear that there are some differences between children conceived using ART and their naturally conceived counterparts. Compared with natural conceptions, ART-conceived children are ~50% more likely to be born small for gestational age (SGA, birth weight <3rd centile for gestation)[2–4] and are ~30–40% more likely to be born with a birth defect.[5,6] Although there is now agreement about these figures, the biological reasons for the observed differences have not been adequately addressed. Possible explanations include an effect of infertility *per se*, or one of the many technical variables associated with ART, such as ovarian stimulation, embryo manipulation and embryo culture. It also remains possible that some of the differences are the consequence of epidemiological difficulties, such as a failure to account for confounding variables or ascertainment bias.[7]

Simultaneously, recent years have seen the identification of a specific link between ART and imprinting disorders, a very rare subset of birth defect. Imprinting is the differential expression of genes according to their parent of origin. Most human genes are expressed approximately equally from copies that are inherited from the mother and the father, whereas imprinted genes are only expressed from either the maternal or paternal copy. The other copy is 'silenced' by a mechanism that is epigenetic, involving methylation of DNA and biochemical modification of histone proteins but not changes in DNA sequence. To date, nine imprinting syndromes have been described in humans, but only three have been associated with ART: Beckwith–Wiedemann syndrome (BWS), Angelman syndrome and the recently described hypomethylation syndrome.[8] Importantly, the imprinted loci associated with these rare syndromes account for only a small fraction of the approximately 200 imprinted genes predicted to be present in the human genome,[9] and the role of most imprinted genes in human health is unknown. What is known is that many imprinted genes appear to have a role in growth, including placental, embryonic and postnatal growth.

Drawing on these observations, Kanber *et al*[1] hypothesized that the effects of abnormal imprinting in ART pregnancies might extend beyond rare imprinting syndromes, and that abnormal imprinting might also explain the increased proportion of SGA babies in ART pregnancies. They have looked for molecular evidence of abnormal imprinting in children who were SGA at birth, and who had also been conceived using intracytoplasmic sperm injection (ICSI), a subset of ART that is used primarily to treat male infertility. Methylation patterns at six imprinted loci were studied in a cohort of 19 ICSI-conceived SGA babies, and compared with the methylation patterns from 29 naturally conceived control children who had normal birth weight. Of the six imprinted loci studied, five had previously been associated with rare human imprinting syndromes, (BWS, Russell–Silver syndrome, transient neonatal diabetes mellitus, and uniparental disomy of chromosome 14).

The important (and somewhat reassuring) finding of this study is that no major difference in imprinting was detected between the ICSI-conceived children and controls. This is the first study to systematically search for imprinting abnormalities at multiple loci in a cohort of children conceived using ART and provides preliminary evidence that abnormal imprinting is not a common feature of children conceived using ART. The results are consistent with existing data that rare imprinting syndromes are infrequent in ART-conceived children.[8] The results are also not particularly surprising because five of the six imprinted loci studied are known to cause specific imprinting syndromes, for which there was no clinical evidence in the children studied. Furthermore, the loci studied represent less than 5% of predicted imprinted loci across the genome; a much greater proportion of these loci will need to be studied before definitive conclusions can be drawn.

The role of abnormal imprinting in the placenta also warrants consideration. Kanber *et al*[1] only studied DNA from buccal cells, yet a recent study suggests that placental tissue might be significantly more susceptible to abnormal imprinting compared with embryonic tissue.[10] Given the importance of the placenta to fetal growth, it is possible that decreased intrauterine growth in ART pregnancies could result from abnormal imprinting that is limited to the placenta, with normal imprinting detected in the embryo/child.

Among their cohort, Kanber *et al*[1] did detect a single ICSI-conceived child with abnormal imprinting. This finding is of uncertain significance, particularly given that the child was completely healthy at follow up and showed normal catch up growth. The specific imprinting abnormality detected was hypermethylation on the paternal copy of the 'KCNQ1OT1' locus. Hypermethylation in this context

most likely represents failure of erasure of the previous imprint during spermatogenesis, and might be related to male infertility. Paternal hypermethylation at the KCNQ1OT1 locus has not previously been described in humans; however, the 'opposite' imprinting change at this locus (hypomethylation of the maternal copy) causes the overgrowth syndrome BWS. It is therefore possible that hypermethylation on the paternal copy of KCNQ1OT1 is the cause of SGA in this child. Whether the imprinting change was caused by the infertility and/or the ART procedure can only be speculated.

The study of Kanber *et al*[1] provides interesting data on the possible association between ART conceptions, intrauterine growth and imprinting abnormalities. At present, this field remains in its infancy, and additional new data are eagerly anticipated. What the data of Kanber *et al*[1] do make clear is that if imprinting differences are to be detected between ART and non-ART children, future studies will need to incorporate significantly larger cohorts of ART-conceived children and controls, analyse more imprinted loci, and analyse multiple tissues, including placenta■

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